THE HEREDITY OF UNILATERAL VARIATIONS IN MAN

C. H. DANFORTH
Department of Anatomy, Stanford University, California

Received December 22, 1923

TABLE OF CONTENTS

	PAGE
Frequency of unilateral variations	199
Relation of unilateral to bilateral appearance of the same trait	202
The causal factors involved in unilateral variations	205
Non-appearance of a trait that might have been expected to be present	206
The influence of the two sides	209
The influence of sex	209
SUMMARY	210
LITERATURE CITED	210

FREQUENCY OF UNILATERAL VARIATIONS

The dissection of even a small series of human bodies always reveals a considerable array of parts which show more or less conspicuous deviation from the normal. In some instances the variations probably represent nothing more than fluctuations in size or position, but not infrequently they are of such a character as to suggest something of a different order. Many of the more common ones are well known, and there is considerable information bearing on the frequency of their occurrence, as well as a certain amount of evidence as to their hereditary nature. The question is somewhat complicated, however, by the fact that, in organs which are ordinarily bilaterally symmetrical, a distinct variation often appears in one member of a pair but not in the other. This fact has suggested to some students that such variations are essentially fortuitous, although it is generally conceded that a hereditary manifestation need not necessarily find a bilateral expression. In view of the existing uncertainty in this regard, the status of unilateral variations with reference to heredity and the factors which influence their appearance, or non-appearance, becomes a matter of genetic interest.

Inasmuch as both sides of the body are developed from the same germ-plasm, it might have been expected that abnormalities which are of germinal origin would almost invariably be symmetrical in their expression, and the question naturally arises as to whether or not uni-

lateral variation ordinarily represents anything other than somatic fluctuation. Two methods of attacking this problem suggest themselves: first, a determination of the relative frequency of the unilateral and the bilateral occurrence of individual variant forms; and, second, the accumulation of evidence of the presence or absence of their hereditary transmission. The first part of the analysis can be undertaken statistically, the second must depend upon the records of individual families. The latter are rarely available in adequate numbers when actual dissection is required to establish the facts.

In order to get some preliminary indication of the amount of unilateral variation to be expected in paired structures taken at random, the upper and lower limbs of twenty unselected subjects were carefully followed through an ordinary course of dissection. Except for occasional unavoidable omissions, 157 pairs of structures (90 muscles, 39 blood vessels and 28 nerves) were examined in each case. The total number of individual parts actually inspected was 5916. Among these there were 530, or approximately 9 percent, which deviated appreciably from the norm as described in standard text-books. The distribution of the abnormalities with reference to sides of the body may be summarized as follows:

Pairs of structures which were normal on both sides	2612
Pairs in which the right was aberrant, the left normal	79
Pairs in which the left was aberrant, the right normal	83
Pairs in which both were aberrant and alike	163
Pairs in which both were aberrant but unlike	21
Total number of pairs compared	2958

The summary shows that there were 162 instances in which structures were abnormal on one side and normal on the other, and 163 in which they were abnormal in the same manner and to about the same extent on both sides. The 21 pairs which were abnormal and unlike probably belong in two categories: (1) those in which there was only a quantitative difference in the expression of the trait on the two sides, and (2) those in which two really different variations happened to appear on opposite sides of the same individual. In practice it is often difficult to differentiate between these two classes. If the number in group (1) could be determined, it might properly be added to the 163 cases of similar bilateral abnormalities, while twice the number in group (2) should be added to 162 in order to make up the total of unilateral manifestations. The figures indicate approximate equality in the unilateral and bilateral expression of abnormality, the ratio lying between 0.88 : 1 and 1.25 : 1.

TABLE 1

The frequency of unilateral and bilateral occurrence in a series of twenty variations, and a comparison of the observed numbers with the numbers expected on the assumption that the distribution is a random one.

STRUCTURE AND TYPE OF VARIATION	*a* TOTAL NUMBER OF PAIRS OF STRUCTURES (SUBJECTS) EXAMINED	*b* VARIATION LIMITED TO THE RIGHT SIDE	*c* VARIATION LIMITED TO THE LEFT SIDE	*d* VARIATION PRESENT ON BOTH SIDES OF THE BODY	*e* OBSERVED / EXPECTED NUMBER OF BILATERAL CASES $\frac{ad}{(b+d)(c+d)}$	*f* OBSERVED / EXPECTED RATIOS OF UNILATERAL TO BILATERAL CASES $\frac{e(b+c+2d)-2d}{b+c}$
Palate bone articulating with frontal	450	11	7	22	10.3	33.0
Malar bone partially subdivided	800	2	2	6	7.5	27.0
Medial infraorbital canal present	2700	8	7	3	10.1	15.7
Infraorbital canal subdivided	3028	86	93	54	7.9	11.8
Supracondylar process of humerus present	2929	10	14	3	3.9	4.2
Os centrale carpi lacking	143	1	7	38	3.1	23.0
External tibiale element present	223	7	10	26	4.9	16.8
Sesamoid bone in peroneus tendon	323	4	8	18	10.2	37.8
Sternalis muscle present	717	18	13	17	11.6	23.2
Axillary arch present	153	1	2	11	10.8	82.6
Biceps muscle with three heads	532	26	61	43	3.2	5.3
Palmaris longus muscle suppressed	2270	87	98	236	4.9	14.8
Flexor pollicis longus muscle lacking	59	8	12	13	1.8	2.8
Psoas minor muscle present	406	26	25	175	1.8	7.3
Piriformis muscle divided	499	59	57	73	2.1	3.5
Quadratus femoris muscle absent	240	8	3	8	10.9	23.3
Peroneus tertius muscle absent	538	16	18	20	7.8	15.8
Plantaris muscle absent	542	9	21	31	8.1	22.7
Carotid artery with "candelabra" division	175	3	6	29	4.5	27.0
Obturator artery arising from epigastric	167	20	28	27	1.7	2.5
Totals	16,904	410	492	853	8.0	21.2

There is no reason to suppose that the material was particularly unusual in either the number or the distribution of its abnormalities.

The frequency of the unilateral and bilateral appearances of certain particular variations is shown in the accompanying table, the data for which have been drawn largely from the reports listed at the end of this paper. To be available for inclusion in the table it was necessary that the record show in each instance (a) the total number of unselected subjects examined; (b) the number of these in which the particular trait under consideration appeared on the right side but not on the left; (c) the number in which it appeared on the left side only; and (d) the number in which it was present on both sides. This necessary requirement renders unavailable many published records, which do not happen to be presented in such form as to be of use for this particular study, but despite this handicap, the absolute number of usable data is rather extensive. For purposes of the present analysis twenty different structures are considered, and the available figures are combined without reference to sex or race. In this material the incidence of variation is 7.4 percent and the ratio of unilateral to bilateral manifestations is 1.06 : 1, which is in fair agreement with the above-mentioned findings for a larger number of structures in a smaller number of bodies. But when individual types of variation are considered separately there is found to be a considerable range, both in the general incidence and in the ratio of unilateral to bilateral cases.

RELATION OF UNILATERAL TO BILATERAL APPEARANCE OF THE SAME TRAIT

Owing to the high frequency of unilateral variations it might be surmised that they are due to environmental influences, or to somatic fluctuations, and that bilateral cases represent chance simultaneous occurrences of the same variation on both sides. This hypothesis can be tested statistically, although there may be some question as to what are the best criteria to employ for the purpose. In any event, if these occurrences may be classed under the head of chance phenomena, the number of bilateral cases of each type should bear some definite relation to the number of unilateral cases of the same type. When in a given sample, a (see columns a to d. in table 1), there are $b+c$ cases of a unilateral variation, the hypothetical total number of right-side cases may be reckoned as $b+d'$ and of left-side cases as $c+d'$. The expected value of d' can then be determined on the basis of a random distribution of the anomaly, and the agreement between observation and expectation tested

by a comparison of d and d'. In the first entry of the table the expected number of bilateral cases according to this procedure is $d' = \frac{(11+d')(7+d')}{(450)^2} \times 450 = 0.178$. The difference between d and d', in this case about 20, is the excess of observed over expected cases of bilateral manifestation of the anomaly. The ratio between the two is about 123 : 1. While this is an essentially logical procedure it has the defect of ignoring the actual value of d in the first part of the calculation, a matter of importance when the numbers dealt with are small.

An alternative method which does not have this defect is to take the true incidence of right-side and left-side cases as $\frac{b+d}{a}$ and $\frac{c+d}{a}$, respectively. Then the expected number of bilateral cases (d') becomes $a \times \frac{(b+d)\ (c+d)}{a^2} = \frac{(b+d)\ (c+d)}{a}$. Dividing the observed number by the expected number gives $\frac{ad}{(b+d)\ (c+d)}$, the ratio of the observed to the expected number of bilateral cases. This ratio, e, is recorded in the sixth column of the table and gives an index of the tendency to bilaterality exhibited by each anomaly. In all cases investigated this value is more than one. If presence of a variation on one side were to inhibit its appearance on the other, e would take a fractional form. The reliability of the index depends on the total number of subjects and the likelihood of a "normal" distribution of the observed number of cases. When the probable error of the observed number of anomalies $(b+c+2d)$ in a total of $2a$ parts (considering both sides) is determined, two values are obtained which differ from the observed value by $-x$ and $+x$, respectively. The most probable values of d' on the basis of these figures lie between $\frac{(b+c+2d-x)^2}{a}$ and $\frac{(b+c+2d+x)^2}{a}$, being grouped asymmetrically around the value obtained directly from the observed figures. In this grouping the chances are slightly over 3 : 1 that the true value does not lie above the upper figure, which may be called d'', and about 10 : 1 against its being $2(d''-d')$. It seems unnecessary to compute these values in each case, and the summary in table 2 will suffice to indicate the figures which may be regarded as statistically significant to the extent that the chances are 10 : 1 against a value of e as large as or larger than the one indicated.

TABLE 2

INCIDENCE OF THE ANOMALY IN PERCENT OF TOTAL NUMBER OF PARTS ($2a$)	VALUES OF e WHICH THEORETICALLY SHOULD NOT BE EQUALED OR EXCEEDED OFTENER THAN ONE TIME IN TEN		
	When $a=100$	When $a=500$	When $a=1000$
0.1	11.5	4.6	3.2
1.0	3.3	1.9	1.6
5.0	1.9	1.4	1.3
10.0	1.6	1.3	1.2
20.0	1.4	1.2	1.1

For some purposes the value e might not be the most satisfactory one since it tends to give more weight to the number of abnormalities than to their observed distribution. Given a certain number of variations in $2a$ parts it may be desirable to compare the observed ratio of bilateral to unilateral cases with the expected ratio between these two classes. This can be done readily when e has been determined. For, the expected number of bilateral cases being $\frac{d}{e}$, the ratio between bilateral and unilateral instances should be (except when b and c differ greatly in value) $\frac{d}{e} : (b+c+2d)-\frac{2d}{e}=\frac{d}{e(b+c+2d)-2d}$. But the observed ratio is $\frac{d}{b+d}$, and this divided by the expected ratio gives $\frac{e(b+c+2d)-2d}{b+c}$, an expression which would seem to allow proper weight for each factor in the empirical data. This ratio, f, is entered in the last column of table 1.

Tested in this manner the twenty traits listed in table 1 all show what may be regarded as a statistically significant excess of bilateral occurrences. In this respect there is an agreement between the two sets of data, one derived from an investigation of a few traits in many subjects, the other from the study of a much larger number of traits in a small number of bodies. This is probably not a coincidence, but may be assumed to indicate the usual situation in reference to the frequent deviations from normal which are listed in text-books of anatomy under the head of "variations." Stated in other terms, it appears that an index of symmetry[1] is generally considerably higher than an index of modality, whether

[1] In an unpublished paper read before Section H of the A.A.A.S., in 1920, the writer proposed the following indices as useful in dealing with certain data relating to human variation: index of modality (translated into terms of the table), $\frac{100\,(a-b-c-d)}{a}$; index of symmetry, $\frac{100\,(a-b-c)}{a}$.

it be organs or whole individuals that are considered. This can only mean that the causal factors tend to be effective on both sides of the body at once, more often than can be accounted for on the basis of chance.

THE CAUSAL FACTORS INVOLVED IN UNILATERAL VARIATIONS

There would seem to be several possibilities as to the nature of these causal factors. They might be environmental, in the sense that they are due to extrinsic influences affecting development either before or after birth; they might represent somatic mutations, as SUMNER and HUESTES (1921) have suggested in the case of certain variations in Peromyscus; or they might be germinal in the ordinary sense. It is also conceivable that the same phenotype may be produced independently by several different causes. In regard to the first possibility: while it is evident that in postnatal life the environment often plays an important rôle in influencing anatomical structure, it is unlikely that anything outside the embryo itself could directly produce the small but distinct deviations which represent the vast majority of congenital variations, and still less probable that such external factors would so frequently be confined in their effect to a small region on only one side of the body. SUMNER and HUESTES argue to the same effect when they suggest something comparable to bud variation in plants as an explanation of asymmetries in Peromyscus. In the case of some gross malformations there is a certain amount of evidence for pathological causation, but there is no indication that the variations under consideration belong to that class.

Scarcely more probable would seem to be the idea of somatic mutation as a general cause of these small variations, at least in human material. The strong tendency for the traits to be bilateral would indicate that the supposed mutations frequently occur as far back as the fertilized ovum and these cases might be expected to be hereditary. This idea leads to the hypothesis that there are two classes of such variations, those due to mutations in the oösperm, which tend to be bilateral and hereditary, and those due to late mutations, which are unilateral and non-hereditary. But if this were the case the high mutation rate as indicated by unilateral cases would lead to the expectation of a rapid increase in the frequency of the trait due to the mutations that occur early enough to involve the germ-plasm. Such traits as have been studied properly show no indications of such an increase. In short, data from the dissecting room do not, so far as can be ascertained, afford any positive evidence in support of the view that the large number of variations ordinarily met with are due to a series of mutations which occurred during the develop-

ment of each particular individual. It is the study of the heredity of one of these variations, however, which throws the most light on the situation and seems to indicate a class of problems of especial interest to the student of human genetics.

The palmaris longus muscle will serve as an illustration, since it represents a type of variable structure commonly encountered in human anatomy and one which has attracted the attention of anatomists in many countries. The total of recorded data in relation to this muscle is greatly in excess of what could be utilized in table 1 and is adequate to show beyond any reasonable doubt that one of its frequent variations, namely, complete suppression, has a different frequency (a) in different races, (b) in the two sexes of the same race, and (c) on the two sides of the body. It is more often suppressed in the white race than in colored races, in women than in men, and in the left than in the right arm. This variation resembles many others that have been extensively studied in showing a difference of incidence with reference to race, sex and side of the body. Such a peculiarity might incline one against the idea of heredity as a factor in its causation. Nevertheless, suppression of the palmaris longus muscle proves to be hereditary (THOMPSON, BATTS and DANFORTH 1921) with no excess of cases left over to be accounted for by environmental factors or somatic mutation. Moreover, family histories show that parents in whom the muscle is suppressed on one side only, may produce children with it suppressed on the same side, on the opposite side, or on both sides, and parents in whom the trait is bilateral produce some children in whom it is unilateral. The evidence in this case seems to make it perfectly clear that a strictly hereditary trait may show all of the characteristics mentioned above; it may be unilateral or bilateral, and it may show a differential frequency in the two sexes and on the two sides of the body.

NON-APPEARANCE OF A TRAIT THAT MIGHT HAVE BEEN EXPECTED TO BE PRESENT

In the variation just mentioned the trait finds only a unilateral expression in about 44 percent of the cases in which it appears at all, and yet, as indicated above, there is no reason to believe that the unilateral cases are genetically in any way different from those in which the trait is bilateral. It would seem rather that the capacity of the gene to reveal its presence through somatic manifestation is so slight and the balance between the influences that make for the appearance of the trait and those which lead to its non-appearance is so even that in about two-

fifths of the cases the actual manifestation is limited to one side of the body. Since the trait fails now on one side, now on the other, it is to be expected that it will occasionally fail on both sides at once. The expected number of such bilateral failures can be calculated if it is assumed that occurrence or failure on one side does not prejudice the chances for occurrence or failure on the other side. If there is not such prejudice, and there is no indication that there is, the expected number of failures should be the probable number on the basis of a chance distribution. Proceeding on this basis x may represent the total number of individuals in a population, a, who are of such genotypical constitution as ordinarily to manifest a trait. Then b represents the empirically determined number in which the trait fails to manifest itself on the left side only; c, the corresponding number for the right side; and d, the number in which it does not fail on either side. In addition to these there is the unknown group in which the trait fails of manifestation on both sides. This may be represented by y. Then

$$b+c+d+y=x \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The incidence of failures on the right side is $\frac{c+y}{x}$ and on the left $\frac{b+y}{x}$. The incidence of bilateral failures should then be the product of these two values, which by definition equals $\frac{y}{x}$ and this equation may be written

$$(b+y)(c+y)=xy \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

From (1) and (2) it follows that $y=\frac{bc}{d}$, which indicates the theoretical number of individuals in a given group, a, which genotypically belong to the aberrant class but phenotypically appear to be in the normal group. In the case of the palmaris longus data presented in table 1, $y=36$ and $x=457$. This would mean that about 8 percent of the individuals who might show the trait fail to do so. The unilateral cases computed with reference to the value of x is 40 percent and the bilateral cases are 52 percent. It might be expected that if this hypothetical 8 percent (1.6 percent of all individuals) who should fail to show the trait really exist, their presence would be revealed in some instances by the character of the offspring. Two cases which agree with such an expectation have been encountered thus far.

Similar non-appearance of a trait, either unilaterally or bilaterally, is not uncommon in hyperdactyly[2] (e.g., unilateral 25 times in one series

[2] BARFURTH (1914) studying hyperdactyly in the feet and wings of the fowl, records similar results and employs a somewhat similar method of analysis.

of 132 subjects in which the condition of the hands are recorded), zygodactyly and some other ordinary anomalies. The value y, which is easily found by dividing bc by d, or which in terms of percent may be indicated as $\frac{100\ bc}{ad}$, should give a clue to the number of cases of non-appearance which ought to be allowed for in either genetic or statistical studies. Thus, if all cases of hare-lip were hereditary, a failure of appearance should be expected, on the basis of RISHBIETH'S (1912) data, in 18 percent of the cases which would have been genetically capable of showing it. These might erroneously be classed with heterozygous dominants. In the case of the supracondylar process of the humerus, another variation in which there is some evidence of heredity (TERRY 1921), non-appearance might be expected, on the basis of the available figures, to be as frequent as 63 percent of the genotypes. It will be apparent that this non-appearance of a trait is wholly different from recessiveness and needs to be taken into account in dealing with hereditary bilateral conditions whether they be genetically dominant or recessive. Consideration of this factor may help to elucidate some of the numerically aberrant results that are often obtained in certain classes of human pedigrees.

The non-appearance of a trait where it might be expected amounts to "failure of dominance" in some instances and in others to the failure of recessive genes to produce the expected result. This suggests that in this group of traits the factors that are actually being studied are "modifiers." It is possible that all individuals are homozygous for the palmaris longus muscle, the normal presence and arrangement of digits and the ordinary type of most anatomical structures but that some have in addition modifying factors which tend to produce the commonly observed anomalies. Some of these modifying factors, if they may be regarded as such, are relatively potent while others are weak. When a particular type of variation owes its existence to a strong modifier, y will be small and the observed occurrence of the trait close to expectation; when it is due to a weak one, y will be large and difficulty of establishing the presence or absence of heredity greatly increased. Thus, SUMNER and HUESTES (1921) conclude that shifting in the point of attachment of the pelvic arch in Peromyscus is not an hereditary trait; but their figures show some excess of bilateral cases, and when tested on the assumption that the condition might be hereditary, a value is obtained for y such as to indicate that between 17 and 18 percent of "normal" mice may potentially represent the abnormal form. When there is a chance that cases of non-appearance are four times as frequent as those in which the trait does

appear the likelihood of getting good Mendelian pedigrees is tremendously reduced and the elimination of heredity as a factor becomes correspondingly difficult.

THE INFLUENCE OF THE TWO SIDES

Less easily brought into line with current genetic ideas is the observed differential incidence of some hereditary traits with reference to the two sides of the body. The factors that influence symmetry are on the whole so little understood that it is hardly expedient to discuss this question beyond emphasizing again that many traits do show a tendency to appear prevailingly on one side. The vast literature on human variation makes this evident. Not only do certain individual variations affecting paired structures show this characteristic, but also some groupings, particularly of vascular arrangement (BEAN 1904, LIPSCHUTZ 1918). Since several of these variations with a bias for one side have been shown to be hereditary, it is difficult to escape the conclusion that in some way the sides of the body, *per se*, exert an influence in determining whether or not a factor represented in the germ-plasm will succeed in producing its full effect. In the case of unpaired and typically asymmetrical organs, what may be an expression of the same influence is so constant as to have been very little appreciated. The mechanism by which such an influence can be exerted is difficult to understand, but the mere existence of the phenomenon as it is revealed differentially by the two sides suggests caution in attributing modifying, intensifying or diluting effects to specific genes until genetic tests have given evidence of the independent existence of such genes.

THE INFLUENCE OF SEX

In differential sex incidence the situation is probably essentially the same, although the difference in the incidence of many variations is greater between the two sexes than between the two sides in the same sex. Thus, to again cite suppression of the palmaris longus muscle, while the trait is only slightly more frequent on the left side, it is about 50 percent more frequent in the female than in the male. The effect of these influences is cumulative, the greatest frequency being on the left side in women, the least on the right side in men. Yet it may be recalled that there is good evidence that the fundamental cause is a factor which is regularly transmitted by heredity. Other traits show differential sex incidences varying through a wide range of values, which indicates that maleness or femaleness of the zygote is by itself a factor which

influences the appearance or non-appearance of certain traits represented in the germ-plasm. These observations suggest that there may be a fallacy in some of the attempts to explain differences in the sex incidence of certain traits (e.g., club-foot?) on the basis of sex-linkage in the ordinary sense. In any event it is clear that sex also is, *per se*, a factor in determining the relative frequency of the appearance or non-appearance of a trait.

Differences in the racial incidence of particular variations are probably chiefly due to the differential distribution of genes with reference to race. But since there is good evidence that sex, and even side of the body, plays a rôle in determining whether or not a trait shall appear, it is easily conceivable that in a similar way the complex which determines race may also act as a factor. Detailed genetic study of favorable traits, among which hyperdactyly is probably one of the best, should ultimately supply an answer to this question.

SUMMARY

Definite variations are found to occur with an average frequency of from 7 to 9 percent in a series of individual anatomical structures which are ordinarily classed as bilaterally symmetrical. Most of these variations find a bilateral expression in some individuals and only a unilateral expression in others.

Several lines of evidence indicate that both the bilateral and the unilateral cases are commonly germinal in origin, and in some cases their heredity has been established. So far as the evidence goes there is no indication that the unilateral appearance of a variation differs genetically from the bilateral.

There is some evidence that most of these traits may best be considered as due to modifying factors that interact with more fundamental determiners for which the species may be homozygous.

Each type of gene has its peculiar degree of potency, which in most cases is such as to admit of certain failures of the trait to appear. This is of considerable importance in genetic analysis, since not infrequently the non-appearance class may be of appreciable size.

The sides of the body and the sex of the individual act as definite influences in favoring or tending to inhibit the appearance of various traits represented in the germ-plasm.

LITERATURE CITED

ADACHI, BUNTARO, 1910 Beiträge zur Anatomie der Japaner. XII. Die Statistik der Muskelvariation. Zeitschr. f. Morph. u. Anthrop. 12: 261-312.

BARDELEBEN, KARL VON, 1909 Ueber bilaterale Asymmetrie beim Mensch und bei höheren Tieren. Verh. d. Anat. Gesellsch., 23ten Versammlung, pp. 2-72.

BARFURTH, DIETRICH, 1914 Experimentelle Untersuchung über die Vererbung der Hyperdactylie bei Hühnern. Vte Mitteilung. Weitere Ergebnisse und Versuch ihrer Deutung nach mendelschen Regeln. Arch. f. Entwicklungsmech. Organ. **40**: 279-309.

BEAN, R. B., 1905 A composite study of the subclavian artery in man. Amer. Jour. Anat. **5**: 303-328.

CADY, LEE D., 1922 The incidence of the supracondyloid process in the insane. Amer. Jour. Phys. Anthrop. **5**: 35-50.

GRUBER, WENZEL, 1879-1882 Beobachtungen aus der menschlichen und vergleichenden Anatomie. Vols. 1-3. Berlin: A. Hirschwald.

LEDOUBLE, A. F., 1897 Traité de variations du système musculaire de l'homme. Vol. 1, 368 pp.; vol. 2, 516 pp. Paris: Schleicher Frères.

1906 Traité de variations des os de la face de l'homme. 471 pp. Paris: Vegot Frères.

LEWIS, THOMAS, 1912 Polydactylism. Treasury of human inheritance **1**: 10-14.

LIPSHUTZ, BENJAMIN, 1918 A composite study of the hypogastric artery and its branches. Annals of Surgery **51**: 585-609.

NAKANO, TATOSO, 1923 Beiträge zur Anatomie der Chinesen. Die Statistik der Muskelvarietaten. Folia Anat. Japon. **1**: 273-289.

PFITZNER, W., 1895 Beiträge zur Kenntniss der menschlichen Extremitäten. Morph. Arbeiten **4**: 347-570.

PICHLER, KARL, 1917-1918 500 Fälle von Sternalmuskel. Beobachtungen am Lebenden. Anat. Anz. **50**: 339-347.

RISHBIETH, H., 1912 Hare-lip and cleft palate. Treasury of human inheritance **1**: 79-123.

SCHWALBE, G., and PFITZNER, W., 1894 Varietation-Statistik und Anthropologie. Morph. Arbeiten **3**: 459-490.

SUMNER, F. B., and HUESTES, R. R., 1921 Bilateral asymmetry in its relation to certain problems of genetics. Genetics **6**: 445-485.

TERRY, R. J., 1921 The supracondyloid process in the living. Amer. Jour. Phys. Anthrop. **4**: 129-139.

THOMPSON, J. W., BATTS, J. MCC., and DANFORTH, C. H., 1921 Hereditary and racial variation in the musculus palmaris longus. Amer. Jour. Phys. Anthrop. **4**: 205-218.

A STUDY OF DWARFNESS IN WHEAT ACCOMPANIED BY UNEXPECTED RATIOS

L. R. WALDRON

North Dakota Agricultural Experiment Station, Agricultural College, North Dakota

Received December 13, 1923

TABLE OF CONTENTS

	PAGE
INTRODUCTION	212
Original work	216
Offspring of normal plants of family 140.10	218
Offspring of dwarf plants of family 140.10	221
Two other dwarf families	226
Dwarf and normal plants secured in 1922 from phenotypically normal parents	226
Family 149.78	230
Family 149.98	231
Plant 140.11	232
Plant 140.17	234
Results from Red Fife×Kota crosses	235
GENERAL DISCUSSION	237
Origin of the factors	242
SUMMARY	244
LITERATURE CITED	245

INTRODUCTION

Plants markedly deficient in height may suddenly appear and their genetical import claims attention. Plants of this nature have been noted several times in the cereals and also in other groups of cultivated plants. COOK (1915) has classified plants of deficient stature into dwarfs and brachytes. He says, in effect, that a dwarf is a plant markedly deficient in stature and having parts correlated with each other as in the normal plant. A brachytic plant, on the other hand, is one possessing a diminished vertical axis, caused by shortening of the internodes, without a corresponding reduction of other parts. He includes in the brachytic class, for example, most of the "dwarf" or "bush" varieties of peas, beans, squashes and tomatoes, the phenomenon being found in other groups of plants also.

Striking and excellent examples of brachytic dwarfs[1] are found in maize reported upon by different investigators. EAST and HAYES (1911) described such a dwarf, having a height of only 18 inches, in a commercial strain of Stowell's Evergreen sweet corn. The leaves were broad but short, while the plant had the appearance of having been telescoped down upon itself. Satisfactory genetical data on this plant were not secured. Dwarf plants of a brachytic type were found by EMERSON (1911) in three families of maize. Families coming from self-pollinated phenotypically normal plants contained dwarf and normal plants in the ratio of 24 to 107. Later studies by EMERSON and EMERSON (1922) revealed the character inherited as a simple recessive to normal.

KEMPTON (1920) crossed two varieties of maize, designated Chinese and Algerian. In the F_2 generation about one-fourth of the plants were of a pronounced dwarf type. When these were selfed or intercrossed, brachytic dwarfs were uniformly produced. Crosses of these dwarfs with normal plants produced normal F_1 offspring with a simple 3-to-1 segregation in the succeeding generation, the dwarf form being simply recessive. In this case dwarf plants appeared when two very distinct varieties of maize were crossed, but as they also appear in self-fertilized commercial strains, this fact evidently has but little bearing upon the origin of the factor. Further work by KEMPTON (1923) has revealed another maize form, of diminished stature, which he considers a true dwarf, not brachytic. Crosses between his two dwarf forms result in entirely normal plants in the first generation. The genes for the two characters of dwarfness must be located in separate chromosomes.

EMERSON (1916) has made a detailed study of bush and twining beans, from a genetical standpoint. He states that the bush bean differs from the twining bean only in its habit of growth. The bush-bean growth is determinate while that of the pole bean is indeterminate. The bush habit, in contrast to the twining habit of pole beans, is conditioned by a smaller number of internodes and also by a relatively small mean internode length. This latter condition evidently has not been brought about by a general shortening of the axis, but rather by a cessation of axial growth early in the period of growth-rate acceleration, before the period of maximum internode-length formation.

Evidently dwarfness in maize, instanced in the examples cited, belongs to a somewhat different category than the dwarf habit of beans. KEMPTON

[1] While recognizing the validity of the classification laid down by COOK, it is more tenable to recognize brachytic plants as dwarf plants of limited character. This article deals with brachytic plants but they will be referred to as dwarf plants or dwarfs.

found that inheritance of dwarfness in maize was due to a single Mendelian factor with normal height dominant. Likewise, EMERSON found the same method of inheritance in bush and twining beans, and he reviews the work of different investigators who have secured similar results working with dwarf varieties of peas, sweet peas, tomatoes and maize. In investigations with the two types of beans, bush and twining, TSCHERMAK (1912) found a complicated method of inheritance. In order to explain the results he assumed the existence of several factors and also the possible formation of unequal numbers of gametes and of zygotes.

VILMORIN (1913) discussed the inheritance of "dwarf" plants found in two varieties of wheat. These plants were about 80 percent of the normal height of the variety from which they were obtained. Seed of dwarf and normal plants was planted for a number of years. Normal plants always produced normal offspring while dwarf plants produced dwarf and normal offspring in the ratio of about 1:2.35. It is not possible to gather from his paper whether a higher proportion of offspring from dwarf plants failed to come to maturity than offspring from normal plants. His failure to secure homozygous dwarf plants is ascribed by him to the failure of the zygotes to survive. In other words, he evidently was working with lethal factors, similar to the classical case of the yellow coat color of mice described by CUÉNOT and others. The dwarf wheats of VILMORIN differ from the dwarf maize plants of KEMPTON and others already discussed in this paper and differ also from the dwarf wheat and oats to be considered later.

MIYAZAWA (1921) crossed two varieties of barley, finding in the F_2 generation one dwarf plant in a total of 96. This dwarf, like that found by VILMORIN, was evidently an intermediate heterozygote differing by one factor from the normal plant. When the young seedlings were given sufficient protection, small and quite sterile dwarfs developed, evidently homozygous. When the barley cultures were well cared for so that the maximum number of plants developed, a reasonable approximation to the 3:1 ratio was secured.

Comparing the results of MIYAZAWA with those of VILMORIN, one finds the two cases similar, but in the case of barley, lethal factors exerted much less influence than in the case of the wheat. Quite certainly the presence of dwarf and sterile wheat plants in VILMORIN'S cultures would have been noticed. The results of MIYAZAWA are apparently intermediate in a way between those of VILMORIN and the results secured by EMERSON with dwarf maize plants. In the case of the dwarf maize it was possible to secure seed from the homozygous forms and produce homozygous offspring in turn. One hesitates to say that the factors bringing about dwarfness in maize are

lethal in character, although it is obvious that the dwarf maize plant has little chance to compete with the normal plant.

Cases have been found in wheat and oats which are apparently entirely comparable with those found by EMERSON and others in maize, that is, plants occur which are strictly dwarf in character and evidently homozygous. The first worker to report dwarf wheat plants was FARRER (1898), working in Australia. He gives an extended description of the plants, quoted by WARBURTON (1919). He speaks of the plants as "grass clumps," thus indicating the abundance of stools which they develop, the plants only occasionally producing heads. He harvested grain from some of the dwarf plants, the offspring consisting of both normal and dwarf plants, while in one instance the entire offspring consisted of dwarfs. He found the plants appearing only when different varieties of common wheats were crossed, none appearing when one of the parent varieties was *Triticum durum* or *T. turgidum*. The abnormal plants nearly always appeared in the F_2 generation; in one instance the F_1 plants were dwarf, although here one of the parents was the result of a recent hybridization and was probably heterozygous in character. From the information furnished by FARRER it is not possible to determine much about the Mendelian behavior of the dwarf plants.

Plants similar to those found by FARRER were reported by RICHARDSON (1913) from Victoria where he found 28 dwarf plants in a total population of 15,800 of hybrid parentage. The dwarf plants had a height of about nine inches. Less than 50 percent formed heads and only nine percent of them produced grain.

NEETHLING (1917), by crossing two common wheats of normal height, secured only normal plants in the F_1 generation; in the F_2 generation, in the one case where data are given, 8 dwarfs appeared in a total of 31 plants. He considered dwarfness to be brought about by a single recessive factor. It is not explained how such a factor could have been carried in one of the two normal parents without an accompanying inhibiting factor. Aside from the single F_2 result for which data are given, his figures are rather more favorable to a two-factor hypothesis, including an inhibiting factor, than to the one-factor hypothesis suggested.

CUTLER (1919) reported dwarf wheat plants occurring in head-rows selected from Marquis wheat. Evidently dwarf plants were found in more than one head-row and their occurrence was noted during three years, 1914 to 1916. CUTLER states that the plants of lowest stature, about nine inches tall, produced a high percentage of dwarf plants, in some cases 100 percent. Extracted normal plants produced only normal offspring,

while intermediate plants produced dwarfs, intermediates and normals in the ratio of 1 : 2 : 1. No data are given in the article to support these statements. Dwarf plants were found in other varieties, according to CUTLER, the variety Red Fife being specifically mentioned. It is quite certain that the dwarf plants observed by CUTLER became manifest as a result of field crosses of Marquis with other varieties, as it is stated that aside from the appearance of dwarfs, variable characters were present relative to color of chaff, beardedness, shape of kernel, etc. It is reasonable to suppose that the dwarfs which appeared in other varieties likewise made themselves known as a result of field crossing.

WARBURTON (1919) found dwarf oat plants in the Victory variety which were entirely comparable to the dwarf wheat plants discussed above. In one head-row of Victory oats grown at Aberdeen, Idaho, 40 percent of the plants were dwarf in character. These measured not over nine inches in height. Seed saved from the dwarf plants was given to two plant breeders for growing. The complete results indicated that the dwarf character was due to a single Mendelian factor. The 3:1 ratio was approximated very closely. WARBURTON does not indicate that other characters besides dwarfness were concerned, and he does not suggest that the dwarfs which produced dwarf offspring were the result of a field cross.

SAX (1921) crossed Bluestem and Amby wheats, planting 52 F_1 seeds of reciprocal crosses. Apparently the entire F_1 offspring was strictly dwarf, producing no fertile culms. Conditions here are entirely different from those reported in most other cases.

STANTON (1923) has recently reported two cases of dwarf oat plants appearing in the F_4 and F_6 generation, of two crosses. In one instance dwarfness proved to be heterozygous in a monohybrid ratio, while in the other case one entire progeny may have been dwarf, comparable with the dwarf hybrid wheat of SAX.

ORIGINAL WORK

In 1918 the writer made certain crosses between a variety of wheat resistant to *Puccinia graminis*, later named Kota (WALDRON and CLARK 1919), and certain rust-susceptible varieties. All wheats were of the common type, *Triticum vulgare*. While dwarfs appeared in hybrid families of different varieties of the susceptible wheats, those occurring from the combination Marquis × Kota were studied in greatest detail. Dwarf and normal plants are shown in figure 1.

In 1919, four F_1 plants are to be noted resulting from Marquis-Kota crosses of 1918. Three of the plants measured 90 cm in height and one

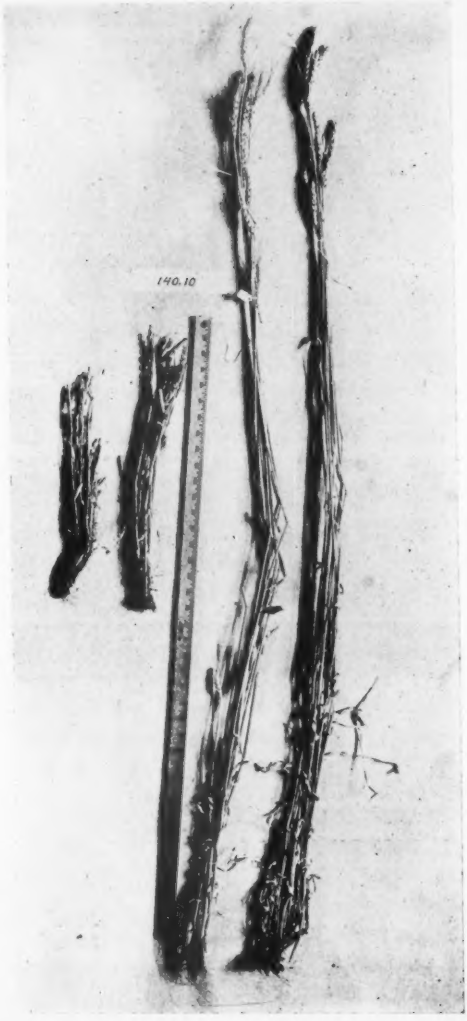

FIGURE 1.—Two plants each of dwarf and normal wheat from family 140.10. The taller dwarf is plant 152.8.

measured 95. In 1920 the four plants produced F_2 offspring as shown in table 1.

TABLE 1

Normals and dwarfs of four Marquis-Kota hybrids.

PEDIGREE NUMBERS	NORMALS	DWARFS	RATIO
140.7	20	5	4.0 : 1
140.10	96	18	5.3 : 1
140.11	53	15	3.5 : 1
140.17	60	9	6.7 : 1
Total	229	47	4.9 : 1

This ratio does not approximate a 3 : 1 ratio and doubt arises immediately as to the simplicity of the dwarf factor carried in the parental form. In crosses between Red Fife wheat and Kota, dwarfs also appeared in the F_2 generation in 1920 as shown in table 2.

TABLE 2

Normals and dwarfs of nine Red Fife-Kota hybrids.

PEDIGREE NUMBERS	NORMALS	DWARFS	RATIO
140.14	38	5	7.6 : 1
140.15	19	2	9.5 : 1
140.20	11	4	2.8 : 1
140.21	85	12	7.1 : 1
140.22	35	1	35.0 : 1
140.27	30	5	6.0 : 1
140.29	23	0	——
140.30	18	0	——
140.31	12	1	12.0 : 1
Total	271	30	9.0 : 1

Only with family 140.20 is there an indication that dwarfness is determined by a single factor. For the remaining cases, complications are indicated except in two cases where dwarf genes may be lacking.

Offspring of normal plants of family 140.10

The greatest amount of data was secured from family 140.10. The average height of the normal plants of the F_2 generation in 1920 was 138.1 cm, with extreme heights ranging from 120 to 159 cm, although there was one plant measuring but 95 cm tall. This plant produced offspring only of normal height in 1921 and was evidently pure for this character. At any rate, no dwarfs were secured from 26 offspring plants.

The dwarfs of 140.10 had an average height of 25.8 cm, the extremes being 9 and 43 cm. The possibility that the dwarf zygotes were less viable than the normal zygotes and thus failed to grow or failed to produce plants living until the close of the growing season, similar to the conditions found by MIYAZAWA (1921), is untenable. For the F_3 generation of 1921 a calculation was made of the percentage of plants coming to maturity, based upon number of seeds sown. As stated, 36 families produced no dwarfs while 42 families produced dwarfs. The percentage of dwarfs per family for the dwarf-carrying families was 18.86 percent. The families producing no dwarfs brought 63.06 ± 1.02 percent of the parent seed to maturity while the families producing dwarfs brought 62.23 ± 1.06 percent of the parent seed to mature plants, or plants which were secured at harvest. If an appreciable number of zygotes had failed to grow at all or to grow sufficiently to be harvested, the fact should have been reflected in a difference of the above means.

Family 140.10 in 1920 produced 96 normals and 18 dwarfs. This obviously approaches a 55 : 9 ratio. If JOHANNSEN'S formula for the probable error of a Mendelian ratio, as modified by BABCOCK and CLAUSEN (1918), be applied to the ratio in question the following relation results:

Phenotype	*Plants*	*Observed ratio*	*Calculated ratio*	*Probable error*
Normal	96	53.9	55	
				± 1.405
Dwarf	18	10.1	9	

The deviation is found to be less than the probable error. The two families, 140.10 and 140.17, taken together, approximate more closely a 55 : 9 ratio than family 140.10, but families 140.7 and 140.11 can not be brought into accord.[2] Certainly the presumption would be that four F_1 plants derived by crossing similar parents would possess genotypes nearly or quite identical relative to the character of dwarfness. The data from family 140.10 are extensive, and with additional available data, the character of the genotype of the F_1 hybrid is foreshadowed.

Of the 96 normal F_2 plants produced in 1920 from 140.10, 78 were planted and 78 families resulted. Of these 78 families, 36 produced no dwarfs in the F_3 generation in 1921 and were thus considered to be dwarf-free.[3] The 42 families which carried dwarfs showed different kinds of segregation indicated in table 3.

[2] Evidence will be presented later to show that the genotype of family 140.11 was perhaps of a nature to produce offspring at the ratio of 55 : 9 (see table 13 and accompanying text).

[3] It will be shown later that this conclusion was not quite true.

TABLE 3

Distribution of dwarf-bearing families relative to normal-to-dwarf ratios found in the F_2 generation of family 140.10 from a Marquis-Kola cross.

FAMILY NO. 149	NORMALS	DWARFS	RATIO
45	47	19	2.5 : 1
50	35	13	2.7 : 1
58	48	16	3.0 : 1
59	42	17	2.5 : 1
61	48	6	3.0 : 1
63	42	16	2.6 : 1
71	54	14	3.9 : 1
77	50	20	2.5 : 1
84	43	13	3.4 : 1
93	51	19	2.7 : 1
103	21	7	3.0 : 1
109	47	12	3.9 : 1
117	50	15	3.3 : 1
Total	578	197	2.9 : 1
	Ratio group, 3.00 : 1		
51	46	11	4.2 : 1
57	56	13	4.3 : 1
70	59	12	4.9 : 1
72	57	14	4.1 : 1
87	38	8	4.8 : 1
Total	256	58	4.4 : 1
	Ratio group, 13 : 3 or 4.3 : 1		
44	56	8	7.0 : 1
54	49	8	6.1 : 1
55	47	9	5.2 : 1
75	41	7	5.9 : 1
81	55	8	6.9 : 1
83	38	6	6.3 : 1
89	53	6	8.8 : 1
90	37	4	9.3 : 1
92	49	9	5.4 : 1
96	53	8	6.6 : 1
9	47	6	7.8 : 1
105	44	7	6.3 : 1
113	73	8	9.1 : 1
115	66	12	5.5 : 1
116	62	11	5.6 : 1
Total	697	117	6.0 : 1
	Ratio group, 55 : 9 or 6.1 : 1		
4	48	4	12.0 : 1
60	55	3	18.3 : 1
64	55	4	13.8 : 1
95	58	3	19.3 : 1
99	67	3	22.3 : 1
100	40	3	13.3 : 1
101	45	4	11.3 : 1
Total	368	24	15.3 : 1
	Ratio group, 15 : 1		
68	61	1	61.0 : 1
104	77	1	77.0 : 1
Total	138	2	69.0 : 1
	Ratio group, 63 : 1		

Summarizing the foregoing table it is seen that evidently there are five kinds of segregation; first, the simple 3 : 1, then 13 : 3, 55 : 9, 15 : 1 and 63 : 1. These ratios are not followed exactly but are very closely approximated. Table 4 presents a summary of table 3.

TABLE 4

NUMBER OF FAMILIES	NORMALS	DWARFS	RATIO
13	578	197	2.9 : 1
5	256	58	13.2 : 3
15	697	117	53.6 : 9
7	368	24	15.3 : 1
2	138	2	69.0 : 1

The average number of individuals per family in the 78 families in the F_3 generation was 59, but in a few of the families the individuals per family were less than half this number. As two of the families had only one dwarf each with the total individuals per family above 60, it is quite possible that certain of the families which showed no dwarfs in 1921 were really not homozygous for normals but showed all normals simply because of too few individuals. As will be seen later, this was found to be true. It is possible to reduce the five ratio groups shown in tables 3 and 4 to four groups by classifying three of the families of the second group with ratio group 3 : 1, and two of the families with ratio group 55 : 9. This does not change the ratios materially and adds to the difficulty of solving the problem.

Offspring of dwarf plants of family 140.10

Although 18 dwarf plants were produced in 1920, only three of them produced seed and only one plant produced seed in any quantity. The dwarf plant, given the number 152.8 (see figure 1), measured 43 cm, produced 105 seeds which were all planted, and in 1921, in the F_3 generation, 76 plants resulted. An even larger percentage of dwarf plants came to maturity from seeds produced by a dwarf than from seed from normal plants. The zygotes surviving during the season were 72 percent, as against 63 percent of plants surviving to maturity from seed producing only normal plants. This indicates again the absence of any lethal action upon the dwarf zygotes. This apparently greater viability perhaps may be explained by the fact that some of the smaller seeds of the dwarf plant were germinated between blotters before being planted out in the field. At any rate there is no evidence of markedly greater failure of seeds producing dwarf plants to develop to maturity, over seeds producing normal plants. Of the 76 resulting plants, 27 were phenotypic normals and 49 were

phenotypic dwarfs. One, or perhaps two, of the dwarfs were perhaps tall enough to have gone into the normal group, but a further test of them showed that they were not normal and produced but a few normal offspring.

The 76 plants of the F_3 generation resulting in 1921 from the dwarf plant 152.8 were distributed as shown in the following tabulation:

Class centers in dm.....	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5
Number of plants.....	3	5	11	16	7	5	1	3	22	3

The bimodality of the foregoing distribution is very striking. In dividing the wheat plants into the two groups, normal and dwarf, the point of nine decimeters was taken. It is seen at once that this is a natural division point and no further argument is needed to sustain it. Plants taller than nine decimeters were considered to be normal and those below were considered to be dwarf. In the foregoing case, trials in 1922 showed that one of the plants, measuring 93 cm, produced mainly dwarf offspring and had a different genotype than the other plants measuring 9 decimeters or more. On the face of the foregoing tabulation 28 normal and 48 dwarf plants resulted from an F_2 dwarf plant. Changing the position of the foregoing plant, changes this distribution to 27 and 49. Omitting this plant from consideration for the present, there were 27 plants which were probably pure for normal height. Seed from 10 of these 27 plants, ranging in height from 100 to 110 cm, was not planted in 1922, and the constitution of these was not known from the behavior of their offspring. However, seed of the remaining 17 normal plants was planted in 1922 and the resulting offspring were all entirely normal.

Of the 49 plants which evidently were wholly dwarf or at least heterozygous for dwarfness, only 38 were represented in the 1922 plantings. Of the 11 plants not represented, eight produced no seed whatever, one produced non-viable seeds, another plant produced four seeds, none of which came to maturity, and one number was lost. The 1922 offspring of the remaining 38 parent plants which were obviously not normal in height (excepting one plant) are shown distributed in table 5. In this table the parent height is given, number of dwarf and normal plants, and the ratio of the two.

A study of table 5 shows in nearly all cases an abrupt falling off of variates immediately below the 95-cm class center. This corresponds closely to the grouping of plants in the parent family. In only one case, family 157.19, is there a continuous distribution from the very obviously dwarf height of 35 cm up to the normal height of about one meter. In this case dwarf plants greatly outnumber normals. With family 157.50 it is

TABLE 5

Distribution of offspring of the F_4 generation from the obviously non-normal plants shown in the foregoing tabulation.

NUMBER	PARENTAL HEIGHT	DWARF	NORMAL	RATIO	CLASS CENTERS IN CENTIMETERS										
					15	25	35	45	55	65	75	85	95	105	115	125
157.																
18	93	87	3	29.0 : 1		4	12	27	24	10	9	1	3			
19	89	92	2	46.0 : 1	1	12	31	20	16	4	5	3	1	1		
20	72	43	23	1.0 : 9		13	16	8	0	6	0	0	2	6	11	4
21	71	56	4	14.0 : 1	4	16	19	11	3	2	1	0	0	4		
22	72	64	4	16.0 : 1	2	22	18	14	7	1	0	0	1	3		
23	72	89	1	89.0 : 1	1	31	34	17	6	0	0	0	0	1		
24	71	39	0	1.0 : 0		9	8	8	13	1						
25	68	34	1	34.0 : 1	1	13	9	6	4	0	0	1	0	0	1	
26	63	36	9	4.0 : 1	3	19	7	5	1	1	0	0	2	7		
27	54	14	3	4.7 : 1		5	2	5	2	0	0	0	0	1	2	
28	60	27	11	2.5 : 1	1	11	10	4	0	1	0	0	3	5	3	
29	63	30	0	1.0 : 0		11	7	10	2							
30	65	44	16	2.75 : 1	2	15	20	4	1	1	1	0	6	8	2	
31	58	Lost in 1921–22														
32	60	27	9	3.0 : 1	3	6	10	8	0	0	0	0	2	5	2	
33	58	15	9	1.7 : 1	3	4	7	1	0	0	0	0	2	7		
34	56	12	5	2.4 : 1	2	4	4	1	1	0	0	0	1	3	1	
35	57	51	12	4.2 : 1	3	28	14	6	0	0	0	0	3	7	2	
36	48	29	1	29.0 : 1	1	9	10	5	4	0	0	0	1			
37	59	24	7	3.4 : 1	1	11	7	3	1	0	1	0	1	3	3	
38	56	19	13	1.5 : 1	2	13	3	1	0	0	0	0	4	6	3	
39	61	17	8	2.1 : 1		9	3	5	0	0	0	0	0	5	3	
40	54	13	2	6.5 : 1	2	5	3	3	0	0	0	0	0	2		
41	50	6	8	0.75 : 1		6	0	0	0	0	0	0	1	1	4	2
42	57	25	1	25.0 : 1	3	8	8	6	0	0	0	0	0	0	1	
43	43	0	0													
44	49	30	10	3.0 : 1	3	10	16	0	0	1	0	0	4	4	2	
45	58	24	0	1.0 : 0		3	11	6	4							
46	52	8	2	4.0 : 1	1	3	1	2	0	1	0	0	0	2		
47	57	22	0	1.0 : 0			8	8	5	1						
48	58	2	1	2.0 : 1	1	0	1	0	0	0	0	0	0	1		
49	48	8	4	2.0 : 1			3	2	2	1	0	0	0	2	2	
50	53	22	6	3.7 : 1	3	7	8	1	0	0	1	2	5	1		
51	46	6	4	1.5 : 1		2	2	0	1	1	0	0	1	2	1	
52	55	3	1	3.0 : 1			2	1	0	0	0	0	0	1		
53	47	3	3	1.0 : 1		1	2	0	0	0	0	0	0	1	1	1
54	46	20	6	3.3 : 1	2	8	8	2	0	0	0	0	3	3		
55	47	27	9	3.0 : 1	1	3	11	8	1	2	0	1	2	5	2	
56	38	12	3	4.0 : 1		4	7	1	0	0	0	0	1	2		
57	38	5	5	1.0 : 1	2	2	1	0	0	0	0	0	0	3	2	

possible the classification of dwarfs as given is not correct and that the three plants at 75 cm and 85 cm are pure for normal height, although

this is unlikely. In a few cases there are plants apparently intermediate between dwarfs and normals, the plant centering at 85 cm, family 157.25, being a striking example. Four of the families (numbers 24, 29, 45 and 47) carry all dwarfs, no plants of normal height being present. It is possible that in one or more of these families, normal plants are absent because of too small numbers but this would hardly be true of all the four families.

TABLE 6

Distributions of table 5 condensed and rearranged to show approach to Mendelian ratios.

FAMILY	NORMALS	DWARFS	RATIO
157.24	0	39	0 : 1
29	0	30	0 : 1
45	0	24	0 : 1
47	0	22	0 : 1
	Ratio group, 0 : 1		
20	23	43	1 : 1.9
26	9	36	1 : 4.0
27	3	14	1 : 4.7
28	11	27	1 : 2.5
30	16	44	1 : 2.8
32	9	27	1 : 3.0
34	5	12	1 : 2.4
35	12	51	1 : 4.3
37	7	24	1 : 3.4
39	8	17	1 : 2.1
40	2	13	1 : 6.5
44	10	30	1 : 3.0
46	2	8	1 : 4.0
48	1	2	1 : 2.0
49	4	8	1 : 2.0
50	6	22	1 : 3.7
52	1	3	1 : 3.0
54	6	20	1 : 3.3
55	9	27	1 : 3.0
56	3	12	1 : 4.0
Total	147	440	1 : 3.0
	Ratio group, 1 : 3		
33	9	15	1 : 1.7
38	13	19	1 : 1.5
41	8	6	1 : 0.8
51	4	6	1 : 1.5
53	3	3	1 : 1.0
57	5	5	1 : 1.0
Total	42	54	1 : 1.3
	Ratio group, 7 : 9		

TABLE 6 (continued)

FAMILY	NORMALS	DWARFS	RATIO
21	4	56	1 : 14.0
22	4	64	1 : 16.0
Total	8	120	1 : 15.0
Ratio group, 1 : 15			
18	3	87	1 : 29.0
25	1	34	1 : 34.0
36	1	29	1 : 29.0
42	1	25	1 : 25.0
Total	6	175	1 : 29.2
Ratio group, (?)			
19	2	92	1 : 46.0
23	1	89	1 : 89.0
Total	3	181	1 : 60.3
Ratio group, 1 : 63			

It is evident from table 5 that a diversity of ratios is present in regard to dwarf and normal plants. How closely the dwarf and normal plants approximate certain well-established ratios is shown in table 6.

One notes that the ratios 1 : 3, 7 : 9 and 1 : 15 are fulfilled exactly, only one additional dwarf being needed in the case of the 1 : 3 ratio. Families 18, 25, 36 and 42 present a ratio quite out of the ordinary and discussion of this will be withheld for the present. The ratio of 1 : 60.3 from families 19 and 23, is to be interpreted as a 1 : 63 ratio; the approximation is satisfactorily close. Reference to table 5 shows that in family 23 there is no possible confusion between the normal plant and the dwarfs. The normal plant was over one meter and none of the dwarfs was as tall as sixty centimeters.

The dwarf plants in 1921, which produced no seed, were among the shortest. Only two plants below 40 cm produced seed. However, one cannot draw the conclusion that the shorter plants were always the more nearly homozygous for dwarfness. Family 57 of 1922, the parent plant of which was but 38 cm tall, produced 50 percent normals and family 56 with the same height of parent plant produced 25 percent normals. One of the families producing all dwarfs, family 24, came from a parent plant 71 cm tall.

Two other dwarf families

In 1919, as stated previously, three dwarf plants of cross 140.10 produced seed. The foregoing discussion has been limited to but one of these plants, having a height of 43 cm. In 1921 there were secured from the two other dwarf plants five dwarfs ranging in height from 24 to 48 cm, the shortest plant producing no seed. A total of 48 seeds from the four remaining plants was planted, 23 plants coming to maturity in 1922.[4] The distribution of the 23 offspring plants is shown in table 7.

TABLE 7

Distribution of normal and dwarf plants in families derived from dwarf plants.

NUMBER	PARENTAL HEIGHT	NORMAL	DWARF	RATIO	CLASS CENTERS IN CM			
					15	25	35	105
157.77	36	1	6	1 : 6.0	2	4	0	1
78	48	2	5	1 : 2.5	0	3	1	2
79	39	0	5	1 : 0.0	1	3	1	0
80	34	2	2	1 : 1.0	0	2	0	2

The foregoing numbers are too meagre to warrant drawing any conclusions, but perhaps a 1 : 3 ratio is indicated, while one family may be pure for dwarf character. Family 157.80 might reveal a 7 : 9 ratio if more plants were available. This ratio might be looked for in this connection as will appear later on in the discussion.

Dwarf and normal plants secured in 1922 from phenotypically normal parents

In 1922 six families were planted of series 149, numbered 64, 65, 74, 77, 78 and 98. Families 65, 74, and 78 are not shown in table 3 as there were no dwarfs in these three families in 1921. In family 64 four dwarfs were found in a total of 59 plants, the ratio being 13.8 : 1 which brought the family into the 15 : 1 ratio group. In 1922 the parent family 64 of 1921 produced 55 families, no dwarfs being present in 28 of these families. For the remaining 27 families normal and dwarf plants were produced as shown in table 8.

The plant 149.64 of table 3 producing the foregoing families appeared to be simply dihybrid in character with two dominant factors responsible for normal height, for out of a total of 59 offspring there were four dwarf plants. In the succeeding generation, shown in table 8, it is evident that conditions in the genotype governing dwarfness are considerably more

[4] In this case the number of plants harvested was only 48 percent of the number of seeds sown. Seeds from dwarf plants are nearly always shriveled and deficient in endosperm, which is usually responsible for less successful growth.

TABLE 8

Distribution, relative to normal-dwarf ratios, of dwarf-bearing families found in the F_4 generation of a family 149.64 derived from a Marquis-Kota cross.

FAMILY	NORMALS	DWARFS	RATIO
156.47	80	25	3.2 : 1
	Ratio group, 3 : 1		
156. 3	59	13	4.5 : 1
9	58	14	4.1 : 1
17	53	14	3.8 : 1
32	60	13	4.6 : 1
44	71	16	4.4 : 1
48	95	23	4.1 : 1
49	96	21	4.6 : 1
Total	492	114	4.3 : 1
	Ratio group, 13 : 3		
156. 5	79	9	8.8 : 1
6	52	8	6.5 : 1
7	38	7	5.4 : 1
8	57	11	5.2 : 1
10	65	9	7.2 : 1
22	59	6	9.8 : 1
24	48	6	8.0 : 1
26	89	10	8.9 : 1
37	71	14	5.1 : 1
38	68	10	6.6 : 1
42	99	16	6.2 : 1
46	98	19	5.2 : 1
53	86	14	6.1 : 1
55	65	8	8.1 : 1
Total	974	147	6.6 : 1
	Ratio group, 55 : 9		
156. 4	55	5	11.0 : 1
31	50	5	10.0 : 1
52	62	4	15.5 : 1
Total	167	14	11.9 : 1
	Ratio group, 15 : 1		
156.11	94	2	47.0 : 1
45	103	1	103.0 : 1
Total	197	3	65.3 : 1
	Ratio group, 63 : 1		

complicated. The following ratios of normals to dwarfs are apparently to be observed in the families resulting from family 149.64, namely, 3 : 1, 13 : 3, 55: 9, 15 : 1 and 63 : 1. Probable errors for these ratios were determined and are shown in table 9.

TABLE 9

Closeness of fit of the observed ratios of table 8 to the calculated ratios.

OBSERVED	CALCULATED	THEORETICAL	ERROR
80	3.045	3	±0.114
25	.952	1	
105	4.000	4	
492	12.990	13	±0.171
114	3.010	3	
606	16.000	16	
974	55.607	55	±0.448
147	8.393	9	
1121	64.000	64	
154	14.754	15	±0.202
13	1.246	1	
167	16.000	16	
197	63.040	63	±0.379
3	.960	1	
200	64.000	64	

In any arrangement like that shown in table 8 some of the more pronounced deviates may be found to have been wrongly placed when another generation is grown. The theoretical and observed ratios are observed to fit very closely. In no case does the observed ratio deviate from the calculated as much as 50 percent more than the probable error. Certain individual family ratios naturally show greater deviation than the mean of several combined families, but most of the individual families are satisfactorily close to their ascribed ratios. The argument might be advanced that from a miscellaneous lot of families, such as shown in table 8, it would be possible to group the families in such a manner as to show almost any series of ratios. The fact of the matter is that the grouping shown in table 8 is the only satisfactory one that can be devised, using ordinary

Mendelian ratios. When the data of table 8 are compared with those of table 3, a striking similarity is observed in that the same ratios are found in both cases, although not in the same proportions. Similarity too close for an accidental occurrence is evident between the genotypes of the grandparent producing the families derived from family 149.64, of table 8, and the F_1 hybrid plant of the original cross.

Family 149.65 produced no dwarfs in 1921 in a total of 61 plants. In 1922, 60 families were grown from the one family of 1921. There resulted 58 families free from dwarfs while two families showed dwarfs as follows:

Family	*Normals*	*Dwarfs*	*Ratio*
156.			
71	82	1	82 : 1
114	58	1	58 : 1
	140	2	70 : 1

Ratio group, 63 : 1

A very simple explanation of the segregation shown in the offspring of family 149.65 seems to be that the plant producing in 1921 the family carrying no dwarfs was really heterozygous for three factors, each responsible for normal height. But if this had been the case, out of 64 plants taken at random from the 1921 family (60 were actually used) eight would show offspring ratios of 63 : 1, 12 would show ratios of 15 : 1, and 6 ratios of 3 : 1. Nothing of the kind occurred, for only two families had a ratio of 63 : 1, while the remainder of the families produced no dwarfs.

Family 149.74 had 57 individuals in 1921, none of which was dwarf. Twenty-five families were grown in 1922 from the parent family and again no dwarfs were produced.

Of family 149.77, 70 plants were grown in 1921 and 20 of these were dwarfs. The ratio of normals to dwarfs was thus 2.5 : 1. In 1922 only 20 families were grown, using normal parent plants taken at random. Of these 20 parent plants, six produced normal offspring plants only. The 14 families carrying both normal and dwarf plants had the offspring in 1922 shown in table 10.

The ratio groups of the offspring of family 149.77 shown in table 10 are the same as those of table 8 except that the 13 : 3 group does not appear. While the families entering into the ratio group 55 : 9 fit less closely to the expected than in some of the other corresponding cases, the fit is acceptable for the two families as a unit, as the deviation with its probable error is 1.147 ± 1.213. It is not desired to enter into a full discussion of these results until later, but attention is called to the four classes

of families listed in table 10. The 20 parent plants, normal in height, were taken from a family ratioed 2.5 : 1 of normal to dwarf plants. If the segregation in the parent family of normals and dwarfs was brought about by a single factor, as is apparently the case, it becomes difficult to explain the 55 : 9, 15 : 1, and 63 : 1 resulting ratios.

TABLE 10

Distribution, relative to dwarf ratios, of dwarf-bearing families found in the F_4 generation of family 149.77 derived from a Marquis-Kota cross.

FAMILY	NORMALS	DWARFS	RATIO
156.			
142	24	8	3.0 : 1
149	50	15	3.3 : 1
152	69	23	3.0 : 1
155	36	11	3.3 : 1
156	31	9	3.4 : 1
157	38	10	3.8 : 1
160	45	15	3.0 : 1
161	17	8	2.7 : 1
Total	310	99	3.1 : 1
	Ratio group, 3 : 1		
148	72	8	9.0 : 1
154	71	12	5.9 : 1
Total	143	20	7.2 : 1
	Ratio group, 55 : 9 (?)		
147	49	3	16.3 : 1
150	79	5	15.8 : 1
Total	128	8	16.0 : 1
	Ratio group 15 : 1		
146	74	1	74.0 : 1
153	86	2	43.0 : 1
Total	160	3	53.3 : 1
	Ratio group 63 : 1		

Family 149.78

Family 149.78 in 1921 carried no dwarfs; all plants were of normal stature. In 1922 seeds of 39 plants were planted and of the 39 families, 32 carried normals only and seven families carried both normals and dwarfs, as shown in table 11.

Families 149.65 and 78 are of course omitted from table 3 as they produced no dwarfs, but with this added knowledge of their genotype evidently they should have been included, presumably in the group ratio 63 : 1, making at least four families segregating 63 : 1.

Family 149.78 and its offspring families show a condition similar to that of family 149.65. Here there is strong evidence, apparently, that three (triplicate) factors are responsible for the normal character, but there is no obvious explanation why nearly all the families carrying dwarfs should

TABLE 11

Distribution, relative to dwarf ratios, of dwarf-bearing families found in the F_4 generation of a family 149.78 derived from a Marquis-Kota cross.

FAMILY	NORMALS	DWARFS	RATIO
156.			
189	95	5	19.0 : 1
	Ratio group 15 : 1		
171	73	1	73.0 : 1
173	97	2	48.5 : 1
180	96	1	96.0 : 1
187	106	2	53.0 : 1
193	92	2	46.0 : 1
199	117	1	117.0 : 1
Total	581	9	64.5 : 1
	Ratio group 63 : 1		

be of the ratio 63 : 1. On the basis of chance eight families out of 64 would throw this percentage of dwarfs and six families out of 39 is not greatly at variance. But at the same time there should be 12 families out of 64 producing normals to dwarfs in the 15 : 1 ratio and six families in the 3 : 1 ratio.

Family 149.98

Family 149.98 in 1921 contained 47 normal plants and 6 dwarfs; thus, in table 1 it is placed in the ratio group 55 : 9. In 1922 15 families were grown from 15 normal plants, obviously taken at random with regard to dwarfness. Of these 15 families seven were free of dwarfs and eight families contained dwarfs as shown in table 12.

The fit in both of these groups is very close. In the first, less than two normal plants need to be added per family to make the ratio exact and the fit in the second group is still closer. Ratios of 13 : 3 and 55 : 9 are not uncommon in Mendelian heredity, and their presence here adds much

weight to a certain part of our general argument. More detailed remarks upon the behavior of these families will be left until the general discussion.

Plant 140.11

In the first part of the discussion of original work, mention was made of a plant, 140.11, which produced 53 normals to 15 dwarfs or at a ratio of 3.5 : 1. The later data secured from the offspring of this plant were less ample than those secured from plant 140.10, but are of decided interest so far as they are available.

TABLE 12

Distribution, relative to normal-dwarf ratios, of dwarf-bearing families found in the F_4 generation of family 149.98 derived from a Marquis-Kota cross.

FAMILY	NORMALS	DWARFS	RATIO
156.			
207	84	17	4.9 : 1
209	70	16	4.4 : 1
210	60	14	4.3 : 1
214	83	22	3.8 : 1
215	84	21	4.0 : 1
Total	381	90	4.2 : 1
	Ratio group, 13 : 3		
202	28	3	9.3 : 1
208	84	14	6.0 : 1
212	88	16	5.5 : 1
Total	200	33	6.1 : 1
	Ratio group 55 : 9		

From the 53 normal plants, 52 were used to produce families for the F_3 generation grown in 1921. Out of the 52 resulting families 23 produced no dwarfs, and 29 families carried dwarfs. This number is compared with families 149.42-119 secured from plant 140.10, in the following tabulation:

Families	149.42–119	149.120–172	
		Observed	*Calculated*
Carrying only normals	34	23	34.5
Carrying normals and dwarfs	44	29	43.5
Total	78	52	78.0

The ratio of families carrying only normal plants to those carrying both normals and dwarfs is seen to be practically identical in the two cases.

The 29 families carrying both normals and dwarfs are shown in table 13.

TABLE 13

Distribution of dwarf-bearing families, relative to normal-dwarf ratios, found in the F_2 generation of plant 140.11 from a Marquis-Kota cross.

FAMILY	NORMALS	DWARFS	RATIO
149			
128	52	23	2.3 : 1
139	56	18	3.1 : 1
141	51	15	3.4 : 1
156	56	15	3.7 : 1
Total	215	71	3.0 : 1
	Ratio group, 3 : 1		
129	28	7	4.0 : 1
131	45	11	4.1 : 1
136	37	9	4.1 : 1
155	50	12	4.2 : 1
161	57	12	4.8 : 1
Total	217	51	4.3 : 1
	Ratio group, 13 : 3		
123	60	8	7.5 : 1
133	48	8	6.0 : 1
138	60	10	6.0 : 1
142	54	9	6.0 : 1
144	50	6	8.3 : 1
158	56	7	8.0 : 1
159	60	7	8.6 : 1
163	47	6	7.8 : 1
167	24	3	8.0 : 1
171	67	13	5.2 : 1
Total	526	77	6.8 : 1
	Ratio group, 55 : 9		
122	51	5	10.2 : 1
126	59	5	11.8 : 1
132	66	4	16.5 : 1
135	58	5	11.6 : 1
145	64	3	21.3 : 1
147	60	4	15.0 : 1
153	46	4	11.5 : 1
162	68	5	13.6 : 1
170	48	3	16.0 : 1
172	61	6	10.2 : 1
Total	581	44	13.2 : 1
	Ratio group, 15 : 1		

With the 29 families four ratio groups are in evidence, namely, 3 : 1, 13 : 3, 55 : 9 and 15 : 1. These correspond to the ratio groups shown in table 3, except that here ratio group 63 : 1 is not represented. The closeness of fit of the different families to their ascribed ratios of table 13 is not quite so good as is found in table 3, although they are very good in both cases. The closeness of fit in the ratio groups of 3 : 1 and 13 : 3 is practically exact, but for the two other groups it is less exact than for the corresponding groups of table 3. Even in these two groups the probable errors calculated for the ratios in the two ratio groups are of the same order of magnitude as the deviations from the observed in comparison with the theoretical ratios. One is entirely justified in dividing up the families into the four ratio groups as indicated. The deviations which are present are not greater than are commonly found in work of this kind.

When a comparison is made in the two tables, relative to the proportionate number of families entering into the two ratio groups, the results are not so satisfactory, but even here analogous comparisons can be made. If one regards either group of families as the basis toward which to work, it is impossible to rearrange the families of the other table in different ratio groups so that the closeness of fit for each ratio group will be improved. If a similarity of distribution of families among the groups in the two tables is to be made uniform, then the character of the ratio groups themselves will be greatly disturbed.

It will be remembered that a fourth generation secured from planting certain of the families of table 3 developed the fact that the ratio group 63 : 1 was represented in two cases in additional families which only came to light by growing another generation. If as large a number of families had been represented in table 13 as in table 3, or if another generation of some of the families apparently carrying all normals had been grown, a more equable chance would have been given for members of the ratio group 63 : 1 to make their appearance.

Without anticipating a discussion of the results as a whole, it is possible to point out that the ratio group 15 : 1 of table 13 is not to be looked for in connection with groups 13 : 3 and 55 : 9. In short, the data from family 140.11 strongly corroborate those from family 140.10, with due respect to certain discrepancies in class groupings, presenting the same sort of a problem for solution.

Plant 140.17

Another plant of the Marquis-Kota cross, number 140.17, produced 60 normals and nine dwarfs in 1920. This is in the ratio of 6.7 : 1 and

suggests a 55 : 9 ratio. In 1921 the seed of 52 normal plants out of the 60 was planted. From this lot of 52 plants, 38 families resulted carrying normal plants only, while 14 families were found to carry both normals and dwarfs. This is an entirely different condition from that obtained with the offspring of the two families 140.10 and 140.11. With only 14 dwarf-carrying families, the numbers are too small to place satisfactorily in different group ratios, but even here it is evident that different classes are in evidence. Without attempting to make a definite grouping, the dwarf-carrying families are shown in table 14, arranged in the order of ratio size.

TABLE 14

FAMILY	NORMALS	DWARFS	RATIO
149.			
211	55	12	4.6 : 1
182	50	10	5.0 : 1
213	54	8	6.8 : 1
207	64	9	7.1 : 1
180	59	8	7.4 : 1
192	73	8	9.1 : 1
185	55	6	9.2 : 1
216	61	6	10.2 : 1
193	71	6	11.8 : 1
173	48	4	12.0 : 1
219	62	5	12.4 : 1
177	33	2	16.5 : 1
194	63	3	21.0 : 1
186	66	3	22.0 : 1

Evidently seven families of this table belong to the ratio group 15 : 1, and possibly two others, 149.185 and 149.192.

Results from Red Fife × Kota crosses

Two families, 140.15 and 140.20, from a cross, Red Fife × Kota, carried normals and dwarfs in 1920 at the rate of 19 normals to 2 dwarfs and 11 normals to 4 dwarfs, respectively, as shown in table 2.

For our purpose these two families may be considered together. In 1921 a total of 30 families were grown from the two parent families numbered 140.15 and 140.20. Out of the 30 families, eight produced both normal and dwarf plants, while the remaining families produced only normal plants. The ratios obtaining in the eight families carrying both normals and dwarfs are shown in table 15.

While the families carrying dwarfs are too few to be classified, they have a value in indicating a diversity of ratios.

This fact also is indicated in the offspring of a cross, 140.1, between the varieties Kota and Red Bobs, made in 1918. The variety Red Bobs, selected in Canada by Doctor SEAGER WHEELER, resulted from a field cross between the Australian variety Bobs and some other variety.

TABLE 15

FAMILY	NORMALS	DWARFS	RATIO
149.			
233	43	9	4.8 : 1
250	39	7	7.0 : 1
238	43	6	7.2 : 1
247	55	7	7.9 : 1
249	44	5	8.8 : 1
240	42	4	10.5 : 1
232	55	5	11.0 : 1
251	37	3	12.3 : 1

In 1920 54 plants of the F_2 generation were produced from the F_1 plant. Twelve of these 54 plants were dwarfs ranging in height from 22 cm to 35 cm. The normal plants were at least one meter high, and most of them considerably taller. The ratio of normals to dwarfs was 4.5 : 1.

In 1921 41 of the 42 F_3 families were grown, of which there were eight which carried both normal and dwarf plants only. Ratios of these eight families are shown in table 16, graded according to size of ratios.

TABLE 16

FAMILY	NORMALS	DWARFS	RATIO
149.			
10	49	9	5.4 : 1
12	68	8	8.5 : 1
5	53	5	10.6 : 1
23	55	4	13.8 : 1
11	59	4	14.8 : 1
14	31	2	15.5 : 1
7	64	4	16.0 : 1
19	64	4	16.0 : 1

In this case probably six families out of the eight belong to the ratio group 15 : 1. If two factors are responsible for the normal condition, as is suggested by the data in table 16, it is not possible to account for the ratios as they stand. In the F_2 generation one dwarf plant out of 15 should

have appeared instead of the two out of nine that did appear. No seed from dwarf plants was planted, so the history of these is lost. Seven out of 15 of the normal plants should have produced families carrying only normal offspring. As a matter of fact, 33 families out of 41 behaved in this manner, which is a much higher percentage than is expected. Of the families which carried both dwarfs and normals, one-half should show the ratio of 3 : 1, and the other half should produce normals and dwarfs in the ratio of 15 : 1. The actual results evidently do not accord at all well with the two-factor hypothesis suggested by the tabulation.

Data secured from the offspring of plants 140.1, 15, 17 and 20 are not sufficient to warrant any attempt at explanation of the facts. The value these data have is to show the complicated nature of the problem and that conditions have arisen which apparently are not amenable to ordinary Mendelian interpretation.

GENERAL DISCUSSION

The families derived from family 140.10 are shown distributed in table 17 with reference to ratio classes. Less ample results from another cross of the same parentage, family 140.11, were similar to those secured from family 140.10. Other results were secured from crosses of other parentage with data too scanty to have a positive bearing.

The ratios shown in table 17 (with the exception of the aberrant and unexplained ratio of 1 : 29) are all of frequent occurrence in Mendelian investigations. Moreover, the approximation of the observed to the calculated results are, in general, very close. While these facts are outstanding, it is nevertheless true that it has not been found possible to postulate a genotypic formula for the F_1 individual which will explain all of the resulting ratios. Any simple hypothesis should be able to accomplish this. The conclusion seems inevitable, then, that the results before us are not amenable to any simple explanation.

The presence of each of the two ratios, 55 : 9 and 63 : 1, according to ordinary Mendelian reasoning, affords ample justification for a three-factor hypothesis. The fact that both ratios are present contrary to any one conventional hypothesis does not lessen at all the probability that three factors are present having to do with normal and dwarf heights. On the contrary, the presence of both ratios acts cumulatively in this respect.

It will be worth while to discuss briefly the hypotheses suggested by the data. First, it may be assumed that three factors were present in the F_1 genotype, each responsible for normal height. The progenies in the F_2 generation should have consisted of normals and dwarfs in a

TABLE 17

Distribution of families derived from family 140.10, carrying normal and dwarf plants, with respect to various ratio classes. The F_1 individual (133.9.1) was of normal height. In each ratio normal plants are placed first.

PARENTAL ORIGIN	RATIO CLASSES											
	1 : 0	3 : 1	13 : 3	55 : 9	15 : 1	63 : 1	0 : 1	1 : 3	7 : 9	1 : 15	(1:29)	1 : 63
F_2 140.10				1(96 : 18)								
F_3 149.42–119 and 152.8... P = 140.10 of 55 : 9 class	36	13	5	15	7	2	0	0	1(27 : 49)	0	–	0
F_4 156.1–55..... P = 149.64 of 15 : 1 class	28	1	7	14	3	2						
156.56–115..... P = 149.65 of 1 : 0 class.	58	0	0	0	0	2						
156.142–161..... P = 149.77 of 3 : 1 class	6	8	0	2	2	2						
156.162–200..... P = 149.78 of 1 : 0 class	32	0	0	0	1	6						
156.201–215..... P = 149.98 of 55 : 9 class	7	0	5	3	0	0						
157.1–57..... P = 152.8 of 7 : 9 class	17	0	0	0	0	0	4	20	6	2	4	2

63 : 1 ratio instead of the 96 : 18 which did result. The theoretical distribution of families among the ratio classes, secured from planting a random sample of normal F_2 plants, in comparison with observed results is shown as follows:

Ratio classes.....	1 : 0	3 : 1	13 : 3	55 : 9	15 : 1	63 : 1
Theoretical.....	46	7	0	0	15	10
Observed.....	36	13	5	15	7	2

Agreement of observed and calculated results is far from satisfactory even ignoring the two ratio groups 13 : 3 and 55 : 9, while if the families in these two groups are distributed among the legitimate classes resulting from the hypothesis the variance would be still more marked, taking into account the disturbances which would result in the fulfillment of the ratio demands. Further, the ratio classes 1 : 3, 1 : 15 and 1 : 63, secured

among the offspring of plant 152.8 can not be explained at all by the duplicate-factor hypothesis without assuming a complete "reversal of dominance."

Another hypothesis suggests itself. One may assume a factor *N*, responsible for normal height, dominant over other factors. Also there is a factor for dwarfness, *D*, which makes itself evident only in the presence of an activating factor, *A*. A little consideration will show that when an F_1 individual is heterozygous for the three factors, the F_2, F_3, and F_4 generations will result as shown in figure 2.

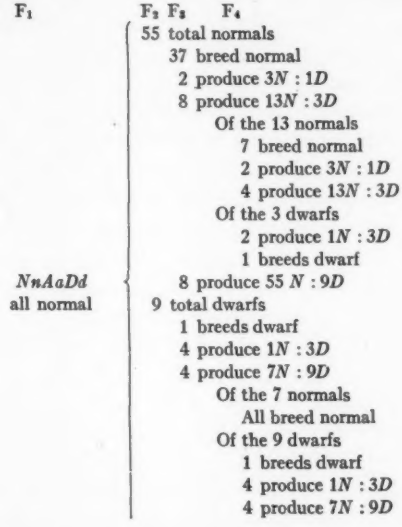

FIGURE 2.—Outline showing expected normal-and-dwarf composition of the offspring for three generations following an F_1 normal individual heterozygous in three factors according to the suggested hypothesis.

Reference to figure 2 shows the ratios 1 : 0, 3 : 1, 13 : 3, 55 : 9, 1 : 3, 7:9, and 0 : 1 of normals to dwarfs to result logically from such a genotype. All of these ratios were found in various families, as is seen by reference to table 17, but the theory does not account for the ratios 15 : 1, 63 : 1, 1 : 15, and 1 : 63. The second theory accounts for a larger number of ratios than the first theory but this helps but little when one recognizes that there are certain outstanding facts which remain unaccounted for.

Assuming the second theory, one striking feature is the great excess of families carrying both normal and dwarf plants with the corresponding

deficiency of families carrying normal plants only. In a total of 169 families presumed to carry only normal plants there were actually only 117 such families, a deficiency of over 30 percent.

If one assumes the second of the above theories as corresponding more nearly to the facts, it is evident that it must undergo some modification to meet the various demands. The necessary modification of the assumed genotype to produce the unexpected ratios of 15 : 1 and 63 : 1 is of a rather profound nature. As the deficient families are those expected to produce only normal offspring it is evident that it is in these genotypes that one must look for modifications. Assuming this theory to be the true one for the time being, any of the numerous normal-breeding genotypes may be taken for illustrative purposes. A zygote of the formula *NNAaDd*, when self-fertilized, would be expected to produce only normal plants. If families are to be produced from this genotype of the ratio of 63 : 1, one of the dominant factors for normal height would have to be modified to become a recessive factor, while the two other dominant factors, for dwarfness and for activation of dwarfness, would have to be modified so that they became responsible for normal height.

It would be a matter of considerable difficulty to prove that the foregoing hypothesis, with its attendant conditions, is correct. In the first place, assuming the theory outlined, there would be 19 different genotypes constituting the normal plants in the F_3 generation, while the dwarf plants would be found to be of four genotypes. This being true, one would be able to pick out by progeny results only a very limited number of known genotypes to be used in genetical analysis. One of these would be *NnAaDd* while another would be *NnAADD*. If individuals carrying these two genotypes were crossed, 14 different genotypes would be expected among a sample of 16 offspring individuals. As only one F_1 individual results from each hybridization, the difficulties of the problem become sufficiently obvious.

Again, if this hypothesis is correct, an attempt at verification must take into account the possibility that a modified instead of an unmodified genotype is being used in test crosses. This may or may not make a difference. Also, in regard to any genotype selected for a test cross, there is a considerable chance that it will undergo modification while in use. As no knowledge of such a modification, aside from a consideration of the progeny, would be available and as the value of the test is dependent upon the distribution of the offspring, it is evident that many of the test crosses would be rendered nugatory. Judging by the number of deficient normal families indicated above, one could expect at least 30 percent of the test crosses to result quite inconclusively.

Because of the uniqueness of the distributions of normals and dwarfs resulting in ratios inexplicable by any one hypothesis, and because of the irregular distribution of families producing the ratio, the testing of the suggested three-factor-modification hypothesis is of much importance in spite of the obvious difficulties. This work is being carried forward at the present time. If the work is carried out successfully one should be able to demonstrate the probable composition of certain of the genotypes. Modification of these in a manner to produce the unexpected ratios as previously indicated could hardly be demonstrated directly, but if modified ratios continued to appear the corollary would be that they arose by reason of the postulated factor modification.

Assuming the genetic composition of the F_3 generation to be as indicated in figure 2, it should not be difficult to isolate individuals with the formula *NNAADD* derived from individuals throwing 3 normals to 1 dwarf, of the formula *NnAADD*. If such an individual be crossed to a sample of individuals derived from a plant producing a 7 : 9 ratio there should result in the F_2 generation families producing normals and dwarfs in the ratios and in the proportions indicated in table 18.

TABLE 18

Calculated distribution of ratios in F_2 families resulting from crossing the tester NNAADD with individuals of the indicated genotypes.

	DWARFS			NORMALS		
	nnAADD	*nnAaDD* *nnAADd*	*nnAaDd*	*nnaaDd* *nnAadd*	*nnAAdd* *nnaaDD*	*nnaadd*
NNAADD (Tester)	3 : 1	½ 3 : 1 ½ 13 : 3	¼ 3 : 1 ½ 13 : 3 ¼ 55 : 9	½ 13 : 3 ¼ 55 : 9	13 : 3	55 : 9

Of the genotypes to be tested shown in table 18, only *nnAADD* and *nnAaDd* would be known directly by the progeny ratios secured through self-fertilization.

Assuming the hypothesis suggested, one sees that if the gametes of the two normal individuals, *nnAAdd* and *nnaaDD* are brought together in hybridization, the entire F_1 progeny will consist of dwarf plants. In this connection it is of interest to recall the results secured by SAX (1921) in crossing two common wheat varieties, Bluestem and Amby. The resulting population of about 50 plants consisted entirely of sterile dwarfs. It would be necessary to assume, in this case, apparently, that the genotype of each variety carried a dominant factor for dwarfness ineffective phenotypically unless acting together in one genotype.

ORIGIN OF THE FACTORS

In regard to the possible origin of the dwarf characters, discussion will be limited to the cereals. There is no evidence, one way or another, whether the factor for dwarfness in maize arose as a mutation at the time of observation or had been already carried in the genotype in a heterozygous condition, as is possible in a population of maize, for a long period without being made noticeable.

In the case of the self-fertilized cereals, wheat, oats and barley, the case is different, for the character in a homozygous condition would find little or no chance of surviving in the somatic form. In the case of the dwarf in oats, cited by WARBURTON (1919), there is little evidence that the dwarf appeared as the result of a cross one or two generations previous to the appearance of the dwarf. He states that while accidental crosses are common with oats in Idaho, all of the plants appearing in the family containing the dwarfs appeared to be of the Victory variety. It seems probable that the dwarf character resulted from a mutation occurring in the parent plant. If the dwarf appeared as the result of accidental crossing between two head-rows of the Victory oats, genotypically unlike, then the difficulty is only pushed back a few generations for a mutation would have to be postulated in one of the pure lines. In this case the mutation would probably have to be of a more complicated character if it were to be carried hidden in a biotype, for a normal homozygous plant carrying a factor or factors for dwarfness must also carry protective genes in the germ-plasm, perhaps in the nature of inhibitors.

It is difficult to believe, in the two cases cited by STANTON (1923), in view of evidence from other investigators, that the crosses made by him four and six generations previous to the appearance of the dwarfs had nothing to do with their becoming visible. At the same time it is difficult to understand the inhibiting mechanism of the genotype protecting homozygous dwarfs or those of a monohybrid ratio, although the phenomenon is not dissimilar from what seems to have occurred in my work where phenotypically normal plants evidently underwent a genotypical modification of a nature to produce dwarfs in place of the expected 100 percent normal offspring as instanced in the apparent deficiency of over 30 percent of normal plants.

In the case of the dwarf wheat plants found by FARRER (1898) and by RICHARDSON (1913) it is impossible to determine the number of the factors responsible for dwarfness. Judging by RICHARDSON'S scant results only very few dwarfs appeared in comparison with normals, thus indicating an apparently complicated condition involving more than one factor.

In the work detailed in this paper, normal-statured plants produced dwarfs of extreme habit as a portion of the progeny. It is difficult to believe that a phenotypically normal plant can carry such factors in its genotype and produce its normal growth. Something like this, however, must occur, for in this investigation some of the offspring of various, apparently normal, plants consisted of dwarfs, indicating that the parent genotype carried factors for dwarfness. We do not know, however, that the presence of the dwarf factor or factors in the genotype of certain of the plants did not have an effect upon the soma. In order to secure accurate information upon this point, it would be necessary to grow families of plants of known genotypes under comparable conditions so that morphological and yield comparisons could be made. A study of this kind would be of importance for it should throw light upon the possible effect upon the soma in maize, brought about by the considerable number of factors known to be carried by the genotype in an ordinarily recessive condition, injurious or even lethal when homozygous.

In this connection a point should not be overlooked which may have a bearing upon the problem. According to the investigation of SAX (1922), common wheat has three times the number of chromosomes found in *Triticum monococcum* and its genotype presumably is hexaploid. If the genotype of common wheat was built up in this manner from a more primitive type of wheat, evidently the factors would be triplicated so that there would be three factors for dwarfness in common wheat. Having three such factors, two of them might become modified in such a manner that they would produce dwarfness only in interacting and not act independently in this capacity.

The difficulty that arises here is to explain how the factors for dwarfness in a recessive condition—evidently they must have been originally in this condition—came to a dominant condition as they are found in the present investigation. There are various difficulties with the hexaploid theory. One is that dwarfness has never been reported in crosses with *durum* wheats.[5] In this group of wheats one would expect to find two factors responsible for dwarfness. It is true, of course, that fewer crosses have been made between *Triticum durum* and *T. vulgare* than between varieties of the latter, and lessened fertility in the progeny of the interspecific crosses must also be considered. Also, speaking generally, one would expect to find many characters in *T. vulgare* based upon three factors in

[5] I take it that the "dwarfs" mentioned by MEISTER (1922) in his crosses of common and *durum* wheat were merely the more heterozygous and sterile forms of something less than normal height found in the F_2 generation.

the genotype, but up to the present time only a very few characters in wheat are determined by three factors. The best known is the red bran color which has been worked out by different investigators.

With the exception of the dwarf oats of WARBURTON (1919) dwarf characters in the self-fertilizing cereals which have been reported, have evidently become manifest only through crossing. According to current Mendelian belief the factor for dwarfness must have existed in the germplasm previous to the cross but was prevented from appearing by the lack of some necessary accessory factor or by the presence of some inhibiting factor or of some dominant normal factor acting inhibitorily. Evidently the dwarf factor arose at some time through mutation. In the case of maize this can happen and the character can be carried along from one generation to another apparently without interference to the continued propagation of the plant. In the self-fertilized cereals, however, such a condition is impossible and the phenotypical dwarf is immediately or soon eliminated. Knowing that factors for dwarfness have arisen in wheat from time to time, basing this knowledge upon results from certain crosses, it is very strange that the character for dwarfness has not made itself immediately manifest. Considering the close study that wheat has had by so many thousands of observers for so many years, it is almost certain that these dwarfs would have been observed and reported if they had come to expression in the soma.

These remarks are merely leading up to the question whether the germplasm of the self-fertilized cereals may not have some sort of a protective or self-regulating device whereby such injurious lethal or semi-lethal mutations as dwarfs are accompanied by other protective mutations. It is well known that maize carries lethal and malforming factors from one generation to another hidden by the dominancy of the normal or "wild" condition. Judging by the great rarity of mutations of this character in wheat, oats and barley, and by the comparative abundance of these characters in maize, one is tempted to believe that they appear less commonly in the normally self-fertilized cereals than in the open-fertilized maize. One of the answers to this suggestion is that in maize these lethal and malforming mutations may have arisen very gradually through hundreds or even thousands of years, and have been carried, perhaps as a continually increasing burden, up to the present time.

SUMMARY

From the original wheat crosses made in 1918, data have been secured for three and four generations. The F_1 individual was of normal height.

In one series the F_3 generation showed families with the following ratios of normal to dwarf plants: 1 : 0, 3 : 1, 13 : 3, 55 : 9, 15 : 1 and 63 : 1. One of the dwarf plants of this series of the F_2 generation produced families in the F_4 generation, with ratios of normal to dwarf plants as follows: 1 : 0, 0 : 1, 1 : 3, 7 : 9, 1 : 15 and 1 : 63. In addition an aberrant ratio of 1 : 29 ± appeared.

These data necessitate some kind of a three-factor hypothesis, but no simple one has been devised to account for a genotype of the F_1 generation producing the various ratios which resulted.

It is suggested that a factor, *N*, is present for normal height dominant over other genotypic conditions. In addition two other factors would be assumed, *A* and *D*, activating and dwarf, resulting in dwarfness when both are present in the absence of dominant *N*. From this there should result 55 : 9 normals to dwarfs, in the F_2 generation. In the F_3 generation normal and dwarf plants should produce families of normals to dwarfs as follows: 1 : 0, 3 : 1, 13 : 3, 55 : 9, 1 : 3, 7 : 9 and 0 : 1. These ratios were secured and also the additional ratios indicated above.

If it be assumed in connection with the foregoing hypothesis, that certain of the genotypes ordinarily producing plants of normal height become so modified that genes for dwarfness are changed to genes for normal, and *vice versa*, the facts may be accounted for.

Work is now in progress to test out the foregoing hypothesis so far as conditions permit. If the hypothesis suggested is the correct one, the labile condition of various genes in the genotype will, in itself, greatly handicap a demonstration of the hypothesis.

Attention is called to the comparative rarity of injurious or lethal characters of this nature, presumably arisen by mutation, found in self-fertilized cereals, in comparison with their comparative abundance in maize. It is suggested, moreover, that a critical comparative study of families of different known genotypes with respect to effect on yield, brought about by presence or absence of dwarf factors in the hybrid wheat under investigation, might throw some light on the effect upon the soma of maize of the various recessive characters normally present, which are lethal or highly injurious when homozygous.

LITERATURE CITED

BABCOCK, E. B., and CLAUSEN, R. E., 1918 Genetics in relation to agriculture. xx+675 pp. New York: McGraw-Hill Book Co.

COOK, O. F., 1915 Brachysm, a hereditary deformity of cotton and other plants. Jour. Agric. Res. **3**: 387–399. *Pls. 53-62.*

CUTLER, G. H., 1919 A dwarf wheat. Jour. Amer. Soc. Agron. **11**: 76–78.

EAST, E. M., and HAYES, H. K., 1911 Inheritance in maize. Connecticut Agric. Exp. Sta. Bull. 167, pp. 5–142. *25 pl.*

EMERSON, R. A., 1911 Genetic correlation and spurious allelomorphism in maize. Nebraska Agric. Exp. Sta. Ann. Rep. **24**: 59-90.

1916 A genetic study of plant height in *Phaseolus vulgaris*. Nebraska Agric. Exp. Sta. Res. Bull. 7, pp. 3-73.

EMERSON, R. A., and EMERSON, S. H., 1922 Genetic interrelations of two andromonoecious types of maize, dwarf and anther ear. Genetics **7**: 203–236.

FARRER, WM., 1898 The making and improvement of wheats for Australian conditions. Agric. Gaz. New South Wales **9**: 152–156. (Also see N. S. W. Dept. Agric. Misc. Pub. 206, 1898.)

KEMPTON, J. H., 1920 Heritable characters in maize. III. Brachytic culms. Jour. Heredity **11**: 111–115. *3 pl.*

1923 Inheritance of dwarfing in maize. Jour. Agric. Res. **25**: 297–322. *5 pl.*

MEISTER, G., 1922 An experiment in studying a cross between distinct species. *Tr. durum* Dest.×*Tr. vulgare* Vill. A preliminary communication. 24 pp. The Saratoff Exp. Sta. [In Russian with English summary.]

MIYAZAWA, B., 1921 Dwarf forms in barley. Jour. Genetics **11**: 205–208. *1 pl.*

NEETHLING, J. H., 1917 A preliminary note on dwarfs appearing in Gluyas Early (wheat) hybrids. South African Jour. of Sci. **14**: 540–547.

RICHARDSON, A. E. V., 1913 Wheat and its cultivation. Victoria Dept. Agric. Bull. 22. pp. 115–116.

SAX, K., 1921 Sterility in wheat hybrids. I. Sterility relationships and endosperm development. Genetics **6**: 399–416.

1922 Sterility in wheat hybrids. II. Chromosome behavior in partially sterile hybrids. Genetics **7**: 513–552. *3 pl.*

STANTON, T. R., 1923 Prolific and other dwarf oats. Dominant dwarfness observed in two oat crosses. Jour. Heredity **14**: 301–305.

TSCHERMAK, E. VON, 1912 Bastardierungsversuche an Levkojen, Erbsen und Bohnen, mit Rücksicht auf die Faktorenlehre. Zeitschr. indukt. Abstamm. u. Vererb. **7**: 81–234.

VILMORIN, PH. DE 1913 Sur une race de blé nain infixable. Jour. Genetics **3**: 67–76. *1 pl.*

WALDRON, L. R., and CLARK, J. A., 1919 Kota, a rust-resisting variety of common spring wheat. Jour. Amer. Soc. Agron. **11**: 187–195. *1 pl.*

WARBURTON, C. W., 1919 The occurrence of dwarfness in oats. Jour. Amer. Soc. Agron. **11**: 72–76. *1 pl.*

LINKAGE OF A DILUTE COLOR FACTOR AND DARK-EYE IN MICE[1]

J. A. DETLEFSEN
The Wistar Institute of Anatomy, Philadelphia, Pennsylvania

AND

L. S. CLEMENTE
University of Illinois, Urbana, Illinois

Received December 24, 1923

TABLE OF CONTENTS

	PAGE
INTRODUCTION	247
Coupling	249
Repulsion	258
SUMMARY	260
LITERATURE CITED	260

INTRODUCTION

In a recent paper, the senior writer described the occurrence of an extremely dilute mutant in the house mouse, which proved to be allelomorphic to both color and albinism (DETLEFSEN 1921). Multiple color-allelomorphs had been described in the guinea-pig (wild or intense color, light dilution, ruby-eyed dilution and Himalayan albinism); in the rat, (wild color, ruby-eyed dilution and albinism); and in the rabbit (wild color, ruby-eyed dilution or so-called chinchilla, Himalayan albinism and complete albinism). WRIGHT (1923) recently added a fifth color mutant (dark dilution) in the guinea-pig series. The paucity of such color allelomorphs in the house mouse stood out in contrast to the other rodents. Although it had been bred by fanciers and investigators for many years, color and albinism were the only two members of the series known. *A priori*, there was no apparent reason why mutations similar to those discovered in other rodents should not occur in mice; and it was therefore of some interest to discover this additional form in this series. Curiously enough, a second

[1] The data were collected by both authors at the College of Agriculture, UNIVERSITY OF ILLINOIS. The senior author is alone responsible for this manuscript and any views or shortcomings it may contain.

additional member of this series in the house mouse appeared at about the same time in the cultures of one of my students (FELDMAN 1923), this last form resembling the ruby-eyed dilute guinea-pig and rat, and the chinchilla rabbit rather more closely than my mutant. The four forms discovered up to the present time in mice represent a set of quadruple allelomorphs and are arranged in the following scale of dominance: wild or intense color (C), ruby-eyed dilution (c^r), extreme dilution (c_d), and complete albinism (c^a). The first is dominant to the other forms, but incomplete dominance is found in connection with the interrelationships among the last three. The experiments described in this paper deal with the third form, extreme dilution (c^d) in relation to intense color (C), albinism (c^a), dark-eye (P), and pink-eye (p). Since it is allelomorphic to intense color, it should likewise show linkage to dark-eye (P). This mutation $\frac{c^d\ P}{c^d\ P}$ occupies, in a scale of dominance, an intermediate position between color and albinism, and therefore gives a coupling series when mated to albinos carrying pink-eye $\left(F_1=\frac{c^d\ P}{c^a\ p}\right)$, but a repulsion series when mated to pink-eyed colored individuals $\left(F_1=\frac{c^d\ P}{C\ p}\right)$.

The original mutant, ♂ 1, and two of his male mutant grandsons, ♂ 3 and ♂ 46, gave rise to 1950 descendants in three types of matings as follows:

TYPE 1. *Matings to pink-eyed brown-spotted females.* From such matings, ♂ 1 produced 8 intense offspring (all wild gray). His grandson, mutant ♂ 3, produced 39 brown agoutis and 36 pink-eyed brown agoutis, and was therefore homozygous in agouti and brown, and heterozygous in dark-eye. His color was fully as dark as that of ♂ 1, thus indicating that the brown and black forms of the mutant may be indistinguishable. The other grandson, ♂ 46, produced 4 wild grays and 4 brown agoutis. The total of 91 intense-colored offspring from these matings proved conclusively that intense color was completely dominant over the extremely dilute color of the mutant. These offspring entered into the experiments on repulsion, for their zygotic formula was obviously $\frac{c^d\ P}{C\ p}$.

TYPE 2. *Matings to albinos carrying pink-eye.* The original mutant ♂ 1, was used in a number of such matings, and produced 57 *mutant* offspring. Such offspring showed pigmented eyes at birth, the pigment being collected largely in a ring around the inner margin of the iris. The

hair was at first white or almost white, but assumed a faint trace of color as the animals grew older. However, dominance was not complete, and the young were as a rule more lightly pigmented than the original homozygous mutants. Their zygotic formula was obviously $\frac{c^d\ P}{c^a\ p}$ and they were used in later experiments on coupling.

TYPE 3. *Matings to homozygous blacks.* The original mutant, ♂ 1, produced from such matings 13 agoutis of intense color. Their formula was $AaCc^d$, and when mated *inter se* gave 40 offspring classified at birth as 29 intense to 11 dilute,—well within reasonable limits of error for a monohybrid ratio. At the age of two weeks, 36 survivors were classified as 17 agoutis : 9 blacks : 10 mutants, where 20.25 : 6.75 : 9.00 is the calculated result on the basis of a 9 : 3 : 4 ratio. The experimental results were therefore consistent with the hypothesis.

These three types of mating also show that the original mutant was homozygous in agouti, black, self-pattern and dark-eye, and was therefore exactly like the wild form from which it arose except that it carried a recessive mutant color gene(c^d) in place of intense color (C).

COUPLING

Male and female double heterozygotes, $\frac{c^d\ P}{c^a\ p}$, which had received color dilution and dark-eye from the mutant parent and albinism and pink-eye from the other parent, were back-crossed to double recessives, $\frac{c^a\ p}{c^a\ p}$. This type of mating produced individuals of four genotypes and two phenotypes as follows:

$c^d\ P$	$c^a\ p$	$c_a\ P$	$c^d\ p$
Dark-eyed dilute	Pink-eyed white		
Parental classes		Crossovers	

All pregnant females were isolated; and since the phenotypes could be and were classified at birth, *postnatal* elimination did not enter as a distorting element in these data on crossing over. One parental class, the dilute mutants, showed pigmented eyes at birth. The other parental class and the crossovers were pink-eyed whites and were always indistinguishable except by a breeding test. Since the crossovers could not be counted

directly, their probable number was estimated in the customary way used in this general type of cross, as, e.g., in the linkage of color and dark-eye in mice, or red-eyed yellow and pink-eyed yellow in rats (CASTLE 1919, DUNN 1920). The usual way is to double the visible parental class (the dark-eyed) and subtract this from the total. The difference given by this indirect method is obviously equal to the difference between the dark-eyed and pink-eyed at birth; for one parental class plus all crossovers minus the other parental class leaves crossovers. The difference obtained by either method approximates the number of crossovers, if there has been no marked *prenatal* elimination. While this conventional procedure assumes that the frequencies of the two parental classes are approximately equal, it also implies tacitly that any random deviations of the two parental classes are always in the same direction, as will be shown later; and this exaggerates the calculated crossover value in either a plus or minus direction giving the appearance of wider deviation in crossover value than one might reasonably expect. The random fluctuations of the two parental classes are, to be sure, just as likely to be opposite in direction. If *prenatal* elimination does take place, the pink-eyed phenotype is more likely to suffer, and the net effect is to reduce the calculated number and percentage of crossovers in the total classified.

Table 1 gives the total results of the coupling series. Taken as they stand, the data indicate coupling of color dilution and dark-eye, as we should expect; but they fail to show as high a crossover value in both sexes or the same degree of consistency which DUNN (1919) found in his

TABLE 1

SOURCE OF DATA	DARK-EYED DILUTE	PINK-EYED WHITE	TOTAL	CALCULATED CROSSOVERS	PERCENTAGE OF CROSSOVERS
F_1 ♂ ♂.....	329	369	698	40	5.73±2.549
F_1 ♀ ♀.....	194	245	439	51	11.62±3.197
Total.....	523	614	1137	91	8.00±1.994

series for color and dark-eye. Our problem, therefore, narrows down largely to discovering whether the difference is due to a functional difference of mechanism or can be attributed reasonably to external and non-genetic factors peculiar to these crosses, or is perhaps associated with the method of determining the number of crossovers. DUNN'S data suggested that coupling in the males (14.28±0.539) and in the females (16.19±0.652) was not significantly different; for the difference (1.91± 0.846) was 2.26 times its probable error. If we use in our data the *same*

formula for the probable error of a calculated crossover value, the males (5.73 ± 0.593) and the females (11.62 ± 1.032) *appear* to differ by 5.89 ± 1.190 with respect to their crossover values;—from which we might conclude that the zygotes classified cannot be regarded as random samples of the same type of population without serious objections and special explanations, for the difference is 4.95 times its probable error. However, our data do not prove a functional difference between the sexes. The routine determination of probable errors of such calculated crossover values by the usual formula, $E = K\sqrt{\frac{pq}{n}}$, must not be taken too seriously, since the use of this formula in these cases rests on an erroneous assumption. There are two ways of determining whether the results from F_1 males and F_1 females in table 1 are significantly different, namely:

(1) We may disregard the question of hypothetical crossover values, and simply ask ourselves whether the percentages of dark-eyed are significantly different, i.e., whether the ratios of $\frac{329}{698}$ and $\frac{194}{439}$ can be regarded as random samples of the same type of population. The F_1 males gave 47.13 ± 1.27 percent dark-eyed, while the F_1 females gave 44.19 ± 1.60 percent dark-eyed. The difference is 2.943 ± 2.043. It is clear that there is no significant difference, in spite of the fact that the F_1 males and females appear to differ by 5.89 percent in calculated crossover value (2×2.943 percent for obvious reasons).

(2) If we prefer to discuss the results in terms of linkage values, we cannot use the usual formula for the probable error of simple sampling, $E = K\sqrt{\frac{pq}{n}}$. (HALDANE'S (1919) formula for the probable error of a linkage value is the same well-known formula expressed in different terms.) The conventional method of arriving at a calculated crossover value in this type of cross has a peculiar consequence which cannot be neglected, for it assumes that random deviations of the two parental classes lie in the same direction. The effect is to exaggerate wide deviations in the calculated crossover value, giving wider deviations than we might at first suspect. If the frequency of each parental class is r and of each crossover class is s, the distribution is theoretically $r+r+s+s=T$, and the calculated crossover value is $\frac{T-2r}{T} = \frac{2s}{T}$. Now, if through sheer fluctuations of sampling the r dark-eyed become $r \mp n$, then the $r+2s$ pink-eyed become $r+2s \mp n$, since we are dealing with correlated

variables. With a fluctuation of $\pm n$ individuals, therefore, the proportion of calculated crossovers becomes $\frac{2s \mp 2n}{T}$, and the calculated percentage of crossovers is lowered or raised by $\frac{2n}{T} \times 100$. This can perhaps be illustrated best if we assume purely for expositive purposes an arbitrary case as follows: We have an average degree of linkage between C and D (color and dark-eye) which gives 14.29 percent crossing over (about the value found for dark-eye and color) and the F_1 ($\widehat{CD}\,\widehat{cd}$) produces 100 offspring in which theory calls for 85.71 of the parental types and 14.29 crossovers distributed in the following proportions:

CD	cd	Cd	cD
6	6	1	1
Dark-eyed	Pink-eyed		

Since the last three genotypes are indistinguishable we actually classify 42.86 percent as one parental class and 57.14 percent as a common phenotype, if we happen to obtain the most probable expectation; and taking the difference we purport to find 14.29 percent crossing over. Now the probable error in a curve of sampling $(\frac{3}{7}+\frac{4}{7})^{100}$ would be ± 3.34; and since it is safe to assume that random deviations of $\pm 3E$ lie within reasonable limits of error in sampling, we might well find ratios of dark-eyed to pink-eyed as low as or as high as $42.86 \pm 3E : 57.14 \mp 3E$; or specifically,

(1) 52.87 : 47.13, i.e., -5.74 percent crossing over

(2) 32.85 : 67.15, i.e., 34.30 percent crossing over.

Thus we might easily arrive at calculated crossover values 20.02 percent ($=\frac{2n}{T} \times 100$, or $\frac{2 \times 3E}{T} \times 100$) below and above the expected 14.29 percent, or a range of about 40 percent in crossover values, simply because the method assumes that the deviations of the two parental classes lie in the same direction. But if we had actually classified crossovers instead of computing their probable number, we should have expected 14.29 percent with ± 7.08 percent as possible deviations lying within similarly reasonable limits of error. Naturally, as the total classified (T) becomes larger, the difference between calculating crossovers and actually classifying them grows less. But it is interesting to note that with numbers even as large as 1000, we might obtain through sheer fluctuations of sampling,

crossover values from 7.95 percent to 20.62 percent, or a range of 12.67 percent, when we calculate the crossover value by this conventional method. Nevertheless, we should expect a range of only 4.46 percent if we were actually able to count the crossovers directly. The method may, therefore, give calculated crossover values which *apparently* lie far outside of reasonable limits of error, if we use the usual formula, which is really inapplicable to cases of this type. Hence, wide deviations do not have the same weight, as if crossovers were counted directly; and we should allow ourselves wider limits of error in dealing with chance deviations in any argument as to what constitutes a significant difference or certainty. If we must allow ourselves wider limits of error in dealing with these calculated crossover values, it follows that the usual formula for the error of a crossover value, $E_c = K\sqrt{\frac{pq}{n}}$, is inappropriate. The roundabout method of obtaining the calculated crossover value has perhaps obscured its real meaning. To double the number of dark-eyed, subtract this from the total, and then convert the difference into a percentage of the total (see CASTLE 1919, p. 31, and DUNN 1920, p. 333) is clearly equal to subtracting the percentage of dark-eyed from pink-eyed. For, if m = the number of dark-eyed, n = number of pink-eyed, and $S = m + n$, then $\frac{S-2m}{S} \times 100 = \frac{100n}{S} - \frac{100m}{S} = \frac{100(n-m)}{S}$. The calculated crossover value is then a simple difference and its probable error is that of a difference. It is an ancient theorem that the standard deviation of a variable, d, which is the difference between two component variables, A and B, is

$$\sigma_d = \sqrt{\sigma_A^2 + \sigma_B^2 - 2r_{AB}\sigma_A\sigma_B} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Since, under ordinary conditions there is no correlation between A and B, the formula is usually shortened to

$$\sigma_d = \sqrt{\sigma^2_A + \sigma^2_B}, \quad . \quad . \quad . \quad . \quad . \quad (2)$$

but it is never safe to assume that $r_{AB} = 0$ in any particular case. In this particular case it happens that the percentage of dark-eyed, $\frac{100m}{S}$, and the pink-eyed, $\frac{100n}{S}$, show perfect negative correlation since they are arithmetical complements. Therefore the probable error of the calculated crossover value in coupling is the error of the difference between $\frac{100n}{S}$

and $\frac{100m}{S}$ using the unreduced formula (1). Since in simple sampling the standard deviations of the proportion of "successes" and of the proportion of "failures" are numerically equal, then in this case in question

$$\sigma_A = \sigma_B; \text{ and}$$

$$\sigma^2_d = \sqrt{4\sigma^2_A} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

In coupling of this type the probable error of a calculated crossover value is therefore twice the probable error of the percentage of either attribute (dark-eye or pink-eye in these experiments).

In view of the foregoing facts, no significant difference can be demonstrated for crossing over in the males and females in table 1. The difference in terms of proportion of dark-eyed is 2.943 ± 2.043 percent ($D \div E = 1.441$); and the difference in terms of calculated linkage values is 5.89 ± 4.089 percent ($D \div E$ is again 1.441, as it should be).

The fact that all three values in table 1 are lower than DUNN'S value for color and dark-eye might suggest a possible general prenatal elimination of my pink-eyed forms. Since, however, the variability in calculated crossover values is so large, our total coupling results (8.00 ± 1.994 percent with 1137 individuals) are not even unquestionably different from DUNN'S results (15.10 ± 1.155 percent with 3331 individuals); for the difference is 7.10 ± 2.30 percent and $D \div E = 3.09$. In linkage studies we have no *a priori* probabilities based on a logical theory, against which we can make our results a test, as in the case of Mendelian ratios; but if we are inclined to assume $\frac{1}{7} = 14.29$ percent crossing over as a "true" value based on DUNN'S total results, then we can say that our numerical results as they stand suggest elimination of the pink-eyed offspring of F_1 males but not of F_1 females.

The elimination of the pink-eyed types would lower the calculated crossover value. In perfectly general terms, if in a total of T zygotes formed and classified the parental classes and crossovers occur in the proportion of $r+r+s+s=T$, then the dark-eyed class $=r$ and the pink-eyed phenotype $=r+2s$, and the crossover value will be $\frac{r+2s-r}{T} = \frac{2s}{T}$. Now, if for any reason pink-eyed are eliminated, so that n of them are not classified at birth, the distribution becomes $r+r+s+s-n=T-n$; and the crossover value becomes $\frac{r+2s-n-r}{T-n} = \frac{2s-n}{T-n}$. It is a simple matter to prove algebraically that the value of $\frac{2s}{T}$ is always greater than $\frac{2s-n}{T-n}$

when $2s$ and T are positive and $2s$ is less than T, as is always the case in this type of distribution. In fact, if n becomes greater than $2s$ (when elimination removes more pink-eyed individuals than there are crossovers) the apparent crossover value becomes negative.

A closer analysis of the data and the circumstances involved in collecting them shows why the offspring of F_1 males might be eliminated more readily than the offspring of F_1 females. In testing the F_1 males by back-crosses we are compelled to use the less vigorous double-recessive females as dams; but in testing the F_1 females with double-recessive males, the vigorous F_1 female hybrids were themselves the dams. This difference in vigor between the two types of dams is reflected in the average size per litter,—in the former case 4.78, but in the latter, 5.48. Such differences in vigor and fertility may have included, as a necessary consequence, some prenatal competition for survival in which the dark-eyed mutants had a distinct advantage over the complementary pink-eyed parental class or the crossovers. The data of our repulsion series (table 3) as well as our general experience in handling the stocks used in these experiments support the contention that the dark-eyed are more vigorous, at least in early postnatal life.

Examining the distribution of calculated crossovers in relation to litter size, we find a distinct difference in the percentage of dark-eyed in litters of less than five and in litters of five or more, when the F_1 males were sires and the double recessives were the dams (table 2). Using the conventional method we should conclude that 37 of the total 40 crossovers

TABLE 2

SOURCE OF DATA	SIZE OF LITTER	DARK-EYED DILUTE	PINK-EYED WHITE	TOTAL	CALCULATED CROSSOVERS	PERCENTAGE OF CROSSOVERS
F_1 ♂ ♂	Less than 5	71	108	179	37	20.67 ± 4.933
	5 and over	258	261	519	3	0.58 ± 2.961
F_1 ♀ ♀	Less than 5	50	58	108	8	7.41 ± 6.472
	5 and over	144	187	331	43	12.99 ± 3.676
	Less than 6	93	120	213	27	12.68 ± 4.584
	6 and over	101	125	226	24	10.62 ± 4.461

in table 1 occurred in the smaller litters, and that the crossover value was 20.67 ± 4.933 percent. But in litters of five and over the number of crossovers was estimated as 3; i.e., only 0.58 ± 2.961 percent crossing over in a total of 519 offspring. The difference between these two crossover values (or between the ratios of $\frac{71}{179}$ and $\frac{3}{519}$) is 3.492 times the probable error.

We are therefore justified in suspecting some selective prenatal elimination, but perhaps not as much as the crossover values themselves might suggest on a cursory examination. In the litters from F_1 females, however, no such striking inequalities occur (see table 2).

As tables 1 and 2 show, the problem of any wide deviation in our ratios from such as DUNN found, is associated largely with litters from F_1 males. The *smaller* litters from the F_1 males may represent either (1) less fertility on the part of the dams, or (2) the residue after rigid prenatal selection, or (3) both in part. If the first condition prevails entirely uncomplicated, there should be no modification of crossover value. Normally small litters would in themselves offer even less occasion for complications due to selective elimination, because, other things being equal, there would be less competition; and we should expect, theoretically perhaps, a fair approximation to the average exchange value found in the case of color and dark-eye. But such smaller litters as represent the residue left after selective prenatal elimination would contain a relatively large number of dark-eyed mutants since these represent the more vigorous zygotes. This would have the effect of apparently lowering the calculated number of crossovers, inasmuch as the difference between the dark-eyed and the pink-eyed at birth is taken as an index of the number of crossovers. The total results found in small litters as a whole would hinge upon the relative frequencies in which these conditions obtain. The *larger* litters may represent (1) those litters which are normally large and have withstood all or almost all elimination by some fortunate sequence of causes; and (2) those litters which remain normally large because they are selected samples containing a larger proportion of the more vigorous and viable dark-eyed mutant zygotes. Had they been samples of the same size but with more pink-eyed zygotes, they would have suffered some elimination of this less viable class. The former conditions would either leave the crossover value unchanged, or lower it according to the amount of elimination. The second condition would lower the crossover value. The net result in larger litters would depend on the balance of these alternative conditions. If our analysis of the conditions which play upon the litter size in these crosses is correct, the result should throw light upon the operation of these conditions. In general, the males gave much more variable results and it will be recalled that they were mated to the weaker double-recessive dams. Dividing their litters into those above and below the average, we find, as stated, no reduction in the smaller litters but a marked lack of calculated crossovers in the larger litters (table 2). It is true that the results in small litters are perceptibly higher than DUNN's

total results; but in view of the method used to calculate crossovers, deviations in crossover value due to sheer sampling can be greater than we might at first expect if we apply the theory of errors to the calculated percentage of crossovers as it stands. The conclusion would be that the smaller litters have suffered at least little or no elimination of pink-eyed zygotes, while the larger litters have undergone some selective process in the form of direct elimination of pink-eyed or the survival of samples with fewer pink-eyed. Either process would lower the calculated crossover value in the larger litters. Somewhere close to the lower limit of relatively large litter size, we should anticipate the cumulative effect of both differential viability and selective sampling, giving the greatest reduction in crossover value. And consistent with this view we found, in litters of six, 103 dark-eyed dilutes to 84 pink-eyed, which gives a calculated crossover value of -10.16 percent, in place of $80 : 107 = 14.29$ percent as we might expect. The result is inconsistent with any present view on crossing over unless elimination or the double effect of two selective processes comes into play at about this point, as hypothesized.

The F_1 females gave more uniform results. The total results (table 1) show a crossover value of 11.62 percent, which is lower than DUNN found for color and dark-eye (16.19 percent). If elimination of the pink-eyed types was the cause of the general decrease in crossover value, it was not as variable a type of elimination as the F_1 males seemed to show (table 2), for the crossover values in litters above and below average litter size do not show such large differences. As stated above, the F_1 dams were more vigorous than the double-recessive females with which the F_1 males were mated, and in this fact we have at least one reason for less intensive elimination.

The variations in calculated crossover values which are due to random sampling are naturally just as likely to occur in excess as in defect; but variations due to direct or differential elimination of the pink-eyed class always give a lowered crossover value. Comparing the two types of modification, it is noteworthy that in any given total (T) of zygotes formed a random fluctuation which gives n pink-eyed less than the calculated $2r+s$ is more effective in reducing the calculated crossover value, than is a direct elimination of n pink-eyed zygotes in the total (T); for $\frac{2s}{T} > \frac{2s-n}{T-n} > \frac{2s-2n}{T}$ under the conditions we find in this type of linkage

experiment. It is therefore difficult to determine in any single experiment whether an individual low crossover value is due to a relatively smaller fluctuation or due to a relatively larger selective elimination. In the light of these observations, we may conclude safely that elimination has played some essential part in reducing our crossover values, because,

(1) All values in tables 1 and 2, with a single exception, are reduced; and

(2) Some of the values are so low they fall outside of reasonable limits of error, and thus suggest selective elimination.

REPULSION

Male and female double heterozygotes, $\frac{c^d\ P}{C\ p}$, which had received the mutant gene (color dilution) and dark-eye from the mutant parent and intense or wild color with pink-eye from the other parent, were back-crossed to double recessives $\frac{c^a\ p}{c^a\ p}$. This type of mating produced individuals of four classes as follows:

$\frac{c^d\ P}{}$	$\frac{C\ p}{}$	$\frac{c^d\ p}{}$	$\frac{C\ P}{}$
Dark-eyed dilute	Pink-eyed intense	Pink-eyed dilute	Dark-eyed intense
Parental		Crossovers	

All pregnant females were isolated as in the coupling series, and the young were classified at birth with respect to eye-color. In this repulsion series, however, (unlike CASTLE'S and DUNN'S repulsion series for dark-eye and albinism *versus* pink-eyed and intense color) our birth records gave no direct or indirect hint of the linkage value. Theoretically, there were two groups of equal frequency, a dark-eyed and a pink-eyed. Each group included a parental and a crossover class. Out of a total of 564 born, 557 were classified at birth as 293 dark-eyed and 264 pink-eyed. The result is not inconsistent with the hypothesis that the two classes are equal at birth, and therefore gives no conclusive evidence of prenatal elimination. If this is so, then our repulsion series differs from our coupling series. The real difference between dark-eyed and pink-eyed may, nevertheless, be greater, and yet partly obscured by a fluctuation of sampling; for, there is always the possibility of a reasonably wide plus deviation in the pink-eyed zygotes at fertilization. The elimination may therefore be greater than is apparent. Furthermore, just as in the case of coupling, the F_1

males were mated to double-recessive females and gave an average of 4.40 per litter (101 litters); but the F_1 females gave 5.43 per litter; although the latter average is based on rather small numbers (21 litters). This difference may again represent prenatal competition and elimination. Of the 557 classified at birth, 83 died within 10 days, of which 61 were pink-eyed and 22 were dark-eyed. Evidently the pink-eyed were in any event less viable in early postnatal life. The remaining 474 were completely identified as to genotype when 10 to 14 days old, and are recorded in table 3.

TABLE 3

SOURCE OF DATA	DARK-EYED DILUTE	PINK-EYED INTENSE	PINK-EYED DILUTE	DARK-EYED INTENSE	CROSS-OVERS	TOTAL	PERCENTAGE OF CROSSOVERS
F_1 ♂ ♂	195	136	22	23	45	376	11.97 ± 1.129
F_1 ♀ ♀	45	33	9	11	20	98	20.41 ± 2.746
Total....	240	169	31	34	65	474	13.71 ± 1.066

The data of table 3 show linkage between color dilution and dark-eye. The results in the males (11.97 ± 1.129 percent) and in the total (13.71 ± 1.066 percent) are not significantly different from those of DUNN working with intense color and dark-eye. The number of offspring from F_1 females was small and the crossover value (20.41 ± 2.746 percent) was large; but the result as it stands is not unquestionably different in a statistical sense from DUNN's result, or our results in the F_1 males. The probable errors in this repulsion series were calculated from the usual formula, and not by the formula used in our coupling series. In DUNN's case of dark-eye and intense color, however, it seems to me that the probable errors should be calculated in the same way as in our coupling series, since the number of crossovers is assumed to be twice the number of dark-eyed observed at birth. Here again one implies that the random deviations in the two crossover classes lie in the same direction. In the case of coupling the difference between the dark-eyed and pink-eyed gives the crossovers. In repulsion this difference gives the non-crossovers. Since the probable error of the percentage of non-crossovers is the same as that of the crossovers, the probable error of the percentage of crossovers may also be looked upon as the error of the difference between the percentage of dark-eyed and pink-eyed, always remembering that they show complete negative correlation, or we may simply look upon the percentage of crossovers in repulsion as twice the percentage of dark-eyed, which is tanta-

mount to saying that the percentage of crossovers is the sum of two equal variables completely correlated. In both cases the probable error is twice the error of either attribute, dark-eyed or pink-eyed.

The total results of table 3 show the marked postnatal elimination of the pink-eyed intense. The 409 parentals were divided into 240 dark-eyed dilute and 169 pink-eyed intense, whereas we expect 204.5 of each class. The deviations are much larger than we would reasonably expect. Curiously enough, if the pink-eyed intense were as numerous as the dark-eyed dilute the percentage of crossovers would be *lowered* to 11.93 percent, which, however, is not an unusual deviation from the theoretical value of about 14.29 percent established by DUNN's data.

In passing, it should be recorded that the pink-eyed form of the mutant heterozygous for albinism $\frac{c^d p}{c^a p}$, was indistinguishable from the true albino. This gives three distinct forms of mice which may have identical somatic characters,—true albinos, the pink-eyed form of black-eyed white (DETLEFSEN 1916), and these pink-eyed dilute-colored.

SUMMARY

Color dilution and dark-eye are linked in mice. The crossover values are in general lower than those found by DUNN for color and dark-eye.

Elimination of the pink-eyed forms takes place, and is probably responsible in part for the reduced crossover values.

A revision of the usual method for calculating probable errors is suggested in cases of this type, where the presumptive total number of crossovers is derived from a single visible parental class or a single visible crossover class.

LITERATURE CITED

CASTLE, W. E., 1919 Studies of heredity in rabbits, rats and mice. Carnegie Inst. Washington Publ. 288, 56 pp.

DETLEFSEN, J. A., 1916 Pink-eyed white mice carrying the color factor. Amer. Nat. **50**: 46–49.

1921 A new mutant in the house mouse. Amer. Nat. **55**: 469–473.

DUNN, L. C., 1919 Linkage in mice and rats. Genetics **5**: 325–343.

FELDMAN, H. W., 1922 A fourth allelomorph in the albino series in mice. Amer. Nat. **56**: 573–574.

HALDANE, J. B. S., 1919 The probable errors of calculated linkage values, and the most accurate method of determining gametic from certain zygotic series. Jour. Genetics **8**: 291–297.

WRIGHT, S., 1923 Two new color factors of the guinea-pig. Amer. Nat. **57**: 42–51.

SPECIES CROSSES IN NICOTIANA, WITH PARTICULAR REFERENCE TO *N. LONGIFLORA*×*N. TABACUM*, *N. LONGIFLORA*×*N. SANDERAE*, *N. TABACUM*×*N. GLAUCA*

WALTER SCOTT MALLOCH AND FRANCES WATSON MALLOCH
University of California, Berkeley, California

Received February 15, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	261
Results of species crosses in Nicotiana	263
Hybrid 23F$_1$19 *Nicotiana longiflora* (22-35 P-1)×*Nicotiana Tabacum* var. "Little Dutch" (22-5 P-4)	266
Nicotiana longiflora×*N. Sanderae*	268
Description of the plants of *N. Sanderae* grown in 1922	273
Nicotiana Tabacum var. *havanensis*×*N. glauca*	278
DISCUSSION OF RESULTS	284
SUMMARY	290
LITERATURE CITED	290

INTRODUCTION

The discussion of reaction systems was stimulated by investigations of species hybrids in Nicotiana. Thus when varieties of *Nicotiana Tabacum* are crossed with *N. sylvestris*, the reaction system of the former is dominant over that of the latter (GOODSPEED and CLAUSEN 1917). When *N. rustica* was crossed with *N. paniculata*, the characters of the first-generation plants blended those of the parents, but the resemblance to *N. rustica* was greater than to *N. paniculata* (EAST 1921). The number of chromosomes in these species, as reported by GOODSPEED (1923), is as follows:

$n=12$, *N. sylvestris*, *N. paniculata*;
$n=24$, *N. Tabacum*, *N. rustica*.

GOODSPEED'S studies on the *N. Tabacum*×*N. sylvestris* cross indicates that in the heterotypic metaphase of the F_1 hybrids, twelve bivalent and twelve univalent chromosomes are present. While his cytological evidence for later generations is not complete, the basic idea of the reaction-system hypothesis still holds.

The complete dominance of any *N. Tabacum* character, whether it be the deep rose-red corolla color of *macrophylla* or the very light pink of "Maryland," and the lack of any interaction of factors in the cross of a white variety like *havanensis* with *sylvestris* give the species hybrid unusual interest. It suggests at once the possibility of using such a hybrid for the equivalent of a back-cross in analyzing varietal crosses in *N. Tabacum.*

The result of the studies on *N. Tabacum* and *N. sylvestris* stimulates others to seek for parallel behavior in other species. Such parallel cases would add to the weight of the reaction-system concept and, incidentally, might be used in analyzing varietal differences. The chromosome theory of heredity developed by MORGAN and others has stimulated similar investigations in Crepis (BABCOCK 1920), maize (LINDSTROM 1923) and other plants. Our interest in Nicotiana began in a research problem started earlier (MALLOCH 1920), which involved a tetramerous character in *N. Tabacum* var. *havanensis.* It was found that on the average this variety produced about eighteen percent of tetramerous flowers on the same plant with the normal pentamerous flowers and that this percentage could be increased by varietal hybridization, as will be shown in a later paragraph. Since the character is quantitative in nature, it is difficult to analyze accurately its manner of inheritance. The utilization of a species hybrid involving a recessive parent like *N. sylvestris* offers a promising method of analysis. This leads to a search for other species which could be used similarly to *N. sylvestris*, but which could be carried on beyond the first generation without the limitations of sterility. The same scheme of analysis should be applicable for such characters as the many shades of corolla color found in *N. Sanderae.*

While numerous crosses have been made in Nicotiana, the results reported upon in table 1 and subsequently discussed might justify a repetition of crosses made previously. An inspection of table 1 will indicate that several species crosses were attempted which involved *N. Tabacum* as one parent. *N. sylvestris* was also used in crosses with other species in the hope that a case might be found in which it behaved as a universal recessive with another polytypic species.

Seed of some of the forms which we wished to use were not available when the crosses were made. A number of new samples of seed were collected by one of us in the fall of 1921, at Urbana, Illinois. A number of successful crosses were made in the spring of 1922 in the greenhouse at Urbana, Illinois. The parents were grown in four-gallon pots, were vigorous, and bloomed well for greenhouse plants. The success of some of the species crosses enumerated in table 1 may be attributed to the more

even temperature found under greenhouse conditions. Under garden conditions at Berkeley, California, many crosses fail, due to a sudden change in temperature. A cross made on a warm day may fail due to a subsequent cold day or night, when the flowers dehisce and fall into the protecting bag. Some of the crosses were grown in the spring of 1923, through the kindness of Professor E. B. BABCOCK, Mr. and Mrs. FROOME T. WATSON, and Mr. and Mrs. JAMES MALLOCH, who provided facilities for conducting the experiment. It seems advisable at this time to make a preliminary report, even though the experiment has not been carried as far as might be desired.

RESULTS OF SPECIES CROSSES IN NICOTIANA

The results of the 1923 tests are summarized in table 1. Other crosses were made, but since they were not tested, we have reserved their discussion for a later date. The seeds were sown on paper-towel germinators which were kept moist but never submerged. As soon as the plants had germinated, they were transferred to pots of soil. The vigorous seedlings succeeded well under this system. The others continued to live in the soil-pots for some time and grew a slight amount (from $\frac{1}{8}$ to $\frac{1}{4}$ of an inch), and finally died. Those which did not germinate consisted mostly of empty shells.

EAST and HAYES (1912) obtained 0 percent germination from *N. Bigelovii*×*N. longiflora*. We obtained fair germination, but the plants died in the seedling stage. A sample of seed was obtained from THE ROYAL BOTANIC GARDENS, Kew, England, labeled *N. persica*, which resembled varieties of *N. rustica*. The germination of the cross, *N. persica* ×*N. Langsdorffii* was poor, but one vigorous plant developed, which resembled *N. persica*. The cross of *N. rustica*×*N. Langsdorffii* did not succeed well, but EAST and HAYES secured very vigorous growth. In the cross between *N. Tabacum*×*N. longiflora*, their hybrid failed to germinate. We were able to germinate one or more plants of this cross, when three different varieties of *N. Tabacum* were involved, as shown in table 1. In the reciprocal cross of *N. longiflora*×*N. Tabacum*, we were able to germinate seed from four different crosses involving three different varieties of *N. Tabacum*. Only two plants of one of these crosses were raised beyond the seedling stage, however. This cross will be discussed in a subsequent paragraph. We crossed *N. longiflora* with *N. sylvestris*, while EAST and HAYES made the reciprocal cross. In both experiments, the seeds failed to germinate. Our crosses of *N. persica* and *N. rustica* with *N. Tabacum* failed to germinate. EAST and HAYES crossed *N.*

TABLE 1

Results of species crosses in Nicotiana.

HYBRID NUMBER	FEMALE PARENT	MALE PARENT	DEGREE OF GROWTH
$23F_1$ 27	*N. Bigelovii* (22–33 P–2)	*N. longiflora* (22–35 P–1)	Died in seedling stage.
$23F_1$ 16	*N. Bigelovii* (22–40 P–2) var. *multivalvis*	*N. Sanderae* (22–53 P–1)	Died in seedling stage.
$23F_1$ 17	*N. Bigelovii* (22–40 P–2) var. *multivalvis*	*N. Sanderae* (22–53 P–2)	Died in seedling stage.
$23F_1$ 18	*N Bigelovii* (22–40 P–2) var. *multivalvis*	*N. Sanderae* (22–53 P–3)	Died in seedling stage.
$23F_1$ 24	*N. longiflora* (22–35 P–1)	*N. Sanderae* (22–53 P–1)	Vigorous; reached maturity.
$23F_1$ 25	*N. longiflora* (22–35 P–11)	*N. Sanderae* (22–53 P–1)	Vigorous; reached maturity.
$23F_1$ 26	*N. longiflora* (22–35 P–11)	*N. Sanderae* (22–53 P–3)	Vigorous; reached maturity.
$23F_1$ 23	*N. longiflora* (22–35 P–11)	*N. sylvestris* (22–1 P–1)	Failed to germinate.
$23F_1$ 19	*N. longiflora* (22–35 P–1)	*N. Tabacum* var. "Little Dutch" (22–5 P–4)	Weak; reached maturity.
$23F_1$ 20	*N. longiflora* (22–35 P–11)	*N. Tabacum* var. "Little Dutch" (22–5 P–13)	Died in seedling stage.
$23F_1$ 22	*N. longiflora* (22–35 P–11)	*N. Tabacum* "Red-flowered type" U.S.D.A. (22–3 P–3)	Died in seedling stage.
$23F_1$ 21	*N. longiflora* (22–35 P–1)	*N. Tabacum* var. "White Burley" (22– P–1)	Died in seedling stage.
$23F_1$ 39, $23F_1$ 40, $23F_1$ 41	*N. persica* (22–50 P–1)	*N. Langsdorffii* (22–51 P–1)	Failed to germinate.
$23F_1$ 42	*N. persica* (22–52 P–2)	*N. Langsdorffii* (22–51 P–1)	One plant developed, resembling *N. persica.*
$23F_1$ 43	*N. persica* (22–50 P–2)	*N. Tabacum* var. "Yellow Pryor" (22–11 P–6)	Failed to germinate.
$23F_1$ 45	*N. rustica* (22–56 P–11)	*N. Tabacum* var. "Yellow Pryor" (22–11 P–6)	Failed to germinate.
$23F_1$ 46	*N. rustica* (22–56 P–1)	*N. Langsdorffii* (22–51 P–30)	Failed to germinate.
$23F_1$ 47	*N. rustica* (22–56 P–11)	*N. Langsdorffii* (22–51 P–1)	Failed to germinate.
$23F_1$ 48	*N. rustica* (22–56 P–12)	*N. Langsdorffii* (22–51 P–30)	One plant germinated, died in seedling stage.
$23F_1$ 3	*N. sylvestris* (22–1 P–1)	*N. alata* var. *grandiflora* (22–52 P–1)	Failed to germinate.
$23F_1$ 4	*N. sylvestris* (22–1 P–1)	*N. alata* var. *grandiflora* (22–52 P–4)	Failed to germinate.
$23F_1$ 5	*N. sylvestris* (22–6 P–7)	*N. alata* var. *grandiflora* (22–52 P–1)	Failed to germinate.
$23F_1$ 7	*N. sylvestris* (22–1 P–1)	*N. Sanderae* (22–53 P–1)	Failed to germinate.

TABLE 1 (continued)

HYBRID NUMBER	FEMALE PARENT	MALE PARENT	DEGREE OF GROWTH
$23F_1$ 8	*N. sylvestris* (22–1 P–1)	*N. Sanderae* (22–53 P–3)	Failed to germinate.
$23F_1$ 9	*N. sylvestris* (22–6 P–17)	*N. Sanderae* (22–53 P–1)	Failed to germinate.
$23F_1$ 10	*N. sylvestris* (22–6 P–20)	*N. Sanderae* (22–53 P–1)	Failed to germinate.
$23F_1$ 11	*N. sylvestris* (22–6 P–23)	*N. Sanderae* (22–53 P–1)	Failed to germinate.
$23F_1$ 12	*N. sylvestris* (22–6 P–23)	*N. Sanderae* (22–53 P–3)	Failed to germinate.
$23F_1$ 13	*N. sylvestris* (22–6 P–30)	*N. Sanderae* (22–53 P–3)	Failed to germinate.
$23F_1$ 91	*N. Tabacum* (22–55 P–1)	*N. alata* var. *grandiflora* (22–52 P–1)	One plant germinated; died in seedling stage.
$23F_1$ 52	*N. Tabacum* var. "Havana" (22–2 P–4)	*N. alata* var. *grandiflora* (22–52 P–1)	Failed to germinate.
$23F_1$ 87	*N. Tabacum* var. "Madole" (22–12 P–3)	*N. alata* var. *grandiflora* (22–52 P–1)	Failed to germinate.
$23F_1$ 88	*N. Tabacum* var. "Madole" (22–12 P–6)	*N. alata* var. *grandiflora* (22–52 P–1)	Failed to germinate.
$23F_1$ 82	*N. Tabacum* var. "Yellow Pryor" (22–11 P–6)	*N. alata* var. *grandiflora* (22–52 P–1)	Failed to germinate.
$23F_1$ 77	*N. Tabacum* var. "Connecticut Broadleaf" (22–70 P–2)	*N. longiflora* (22–35 P–1)	Failed to germinate.
$23F_1$ 51	*N. Tabacum* var. "Havana" (22–2 P–4)	*N. longiflora* (22–35 P–1)	One plant germinated; died in seedling stage.
$23F_1$ 73	*N. Tabacum* var. "Little Dutch" (22–5 P–3)	*N. longiflora* (22–35 P–1)	One plant germinated; died in seedling stage.
$23F_1$ 59	*N. Tabacum* var. "Red-flowered" (22–3 P–3)	*N. longiflora* (22–35 P–11)	Three germinated; died in seedling stage.
$23F_1$ 78	*N. Tabacum* var. "White Burley" (22–9 P–1)	*N. longiflora* (22–35 P–11)	Failed to germinate.
$23F_1$ 81	*N. Tabacum* var. "Yellow Pryor" (22–11 P–2)	*N. longiflora* (22–35 P–11)	Failed to germinate.
$23F_1$ 90	*N. Tabacum* var. "Lacks" (22–21 P–1)	*N. Sanderae* (22–53 P–1)	Failed to germinate.
$23F_1$ 75	*N. Tabacum* var. "Little Dutch" (22–5 P–14)	*N. Sanderae* (22–53 P–1)	Failed to germinate.
$23F_1$ 89	*N. Tabacum* var. "Madole" (22–12 P–5)	*N. Sanderae* (22–53 P–1)	Failed to germinate.

rustica with *N. Tabacum* and secured a low percentage of germination and very vigorous plants.

In the crosses just discussed, involving *N. Bigelovii*×*N. longiflora* and *N. longiflora*×*N. sylvestris*, there is little doubt of the high degree of incompatibility between the species concerned. In the cases where the

two experiments failed to agree, we must attribute some other cause to the one which failed, such as varietal differences. One of our associates has cultures demonstrating marked differences in *N. Tabacum*×*N. glauca* crosses, depending upon the particular *Tabacum* variety used in the cross. Lack of optimum conditions at the time of seed development and at the time of germination are factors which might affect the crosses.

Taxonomists appear to be confused as to the proper classification of the form known as *multivalvis*. SETCHELL (1912) gives it species rank under the name of *N. multivalvis*, but adds that it is commonly assigned as a variety under *N. quadrivalvis*. GRAY (1876, p. 546) has stated that "*N. Bigelovii* is perhaps the original of it," to which SETCHELL agrees, stating "since it has appeared in the pedigreed cultivation of *N. Bigelovii* in the U. C. B. G." *Multivalvis* appears to be a giant form of *N. Bigelovii* differing in the many-celled indehiscent capsule and in the many-lobed corolla limb. RICKER (1916) has mentioned it under *N. quadrivalvis*. In our experiments, *multivalvis* and *Bigelovii* cross freely and produce fertile hybrids. In table 1, we have listed it under *N. Bigelovii* for convenience.

Hybrid 23 F 19 Nicotiana longiflora (22-35 P−1)×*Nicotiana Tabacum var. "Little Dutch" (22-5 P−4)*

Nicotiana longiflora was obtained from Mr. JAMES JOHNSON of the U. S. DEPARTMENT OF AGRICULTURE. Two plants grown in 1922 and sixty-seven plants grown in 1923 were all of one type. As grown in the open garden at Berkeley in 1923, the plants were from 3 to 4 feet high, with numerous branches, giving the plant a bush-like shape. The stems are bristly-scabrous and slender. The auricles of the leaves extend half-way around the stem, and the leaf blade is distinctly undulate and spatulate to lanceolate in shape. The flowers are about 10 cm long, with a spread of about 4 cm. The corolla is villous, yellowish-violet on the exterior and white on the inner surface. It is salverform in shape, with a slender tube. The filaments of the anthers are adnate for their entire length. The flowers open in the early evening and close during the day.

N. Tabacum var. "Little Dutch" was obtained from Mr. H. A. ALLARD of the U. S. DEPARTMENT OF AGRICULTURE. Eight plants grown in 1922 and twenty-two plants grown in 1923 were all of a uniform type. The plants grew to a height of 5 to 6 feet at Berkeley, California. The stems are viscid-pubescent and stout. The leaves are broadly fusiform, being acute at both base and apex and broad in the middle of the leaf. The

flowers are 8 cm long, with a spread of about 2.5 cm. The corolla is woolly on the outer surface. The color of the inner surface of the corolla is Hermosa Pink,[1] tinged with Deep Rose Pink. The lobes are distinctly acuminate, and the filaments of the anthers are free for their entire length. The flowers remain open both day and night.

The germination of the seed from this cross was poor, being about 25 percent. Only two plants survived, all the rest dying in the seedling stage. Both plants grew slowly and were too weak to risk planting in the field. The leaves were at first a light green, with numerous yellow spots and patches. The plants remained in the rosette stage until July, when they slowly sent up a shoot. One plant grew more slowly than the other and reached a height of only 12 cm, when a presumptuous gardener threw the plants out. This plant was too weak to be revived. The second plant had reached its full growth and was revived long enough to obtain a few more blooms. This plant reached a height of 50 cm and produced one main shoot with three alternate branches. The stems were stout for the size of the plant, the main stem being 1.5 cm in diameter at the base, and the branches from 0.5 to 0.7 cm in diameter at their bases. The stems were viscid and pubescent, thus resembling the *Tabacum* parent.

The yellow-spotted rosette leaves gradually died off, and the caulescent leaves that developed were of a dark-green color and were apparently without chlorophyll deficiency. The rosette leaves reached a length of 28 cm and a width of 6 cm and were fusiform in shape. The caulescent leaves varied from a fusiform to a linear leaf which tapered abruptly about 5 cm from the stem, leaving a strip of leaf 0.5 cm wide to form a winged petiole flaring slightly at the stem. The lower caulescent leaves reached a length of 18 cm with a width of 2 cm. All of the leaves were of an irregular wavy outline throughout the length of the leaf blade, instead of presenting a smooth curve from apex to base. This irregular outline was not due to undulations, as these were not pronounced, but to different widths of the blade from the midrib to the edge of the leaf.

The flowers remained open day and night as in the *Tabacum* parent. The flowers were about 5.5 cm long, with a spread of about 2 cm. The corolla was woolly without and had a comparatively stout tube. The lobes of the corolla were irregularly notched, giving a slight resemblance to a fringed Petunia. The pistil was the same length as the corolla, but the stamens were only two-thirds as long and were adnate to the corolla for half of their length from the base up. In flowers having one short stamen,

[1] The technical color terms used in this paper are from RIDGWAY (1912).

this one was adnate its entire length. The color of the inner surface of the corolla was Hermosa Pink, tinged with Deep Rose Pink, as in the *Tabacum* parent. The flowers dropped off a day or two after opening. The fertility of 23 F_1 19 might be increased under field conditions, and it might be possible to cross it back on one of the parents. The flowers developed too late to accomplish this successfully in 1923.

LINDSTROM (1918) has reported on a virescent-white corn character, which usually begins as a pure white seedling but under favorable conditions assumes a yellowish-green color, and the plant may finally become a normal green. Under favorable conditions, ripened seed may be obtained from such plants. The virescent-white seedlings behave as a simple recessive to the normal green plants. The virescent-white plants arose as the result of a mutation from normal green plants within a species, while the hybrid 23 F_1 19 was derived from a distinct species cross, both parents being of a normal green color and vigorous. The corn character started with less chlorophyll and reached greater fertility; the tobacco hybrid started with more chlorophyll and had less fertility at maturity. The two cases are somewhat parallel in their behavior, however, as both began in a weak condition, had a slow rate of growth, finally developing the full amount of chlorophyll, and were weak in fertility.

This cross fits into the series of species crosses which range from complete fertility to complete incompatibility, referred to in an earlier article by one of us, on species hybrids in Hordeum (MALLOCH 1921). The genetic aspects will be given fuller attention in the general discussion. The behavior of this hybrid may be summarized as follows:

(1) The hybrid shows a marked reduction in vigor from that of either parent, accompanied by a small amount of albinism, and marked sterility.

(2) The flower color, flower size, flower shape, time of flower opening, habit of growth, and the pubescence of the plant more nearly resemble *N. Tabacum*.

(3) The fringed corolla lobes and the type of leaf are characters not common to either parent.

(4) The attachment of the stamens is an intermediate character between the two parents.

Nicotiana longiflora × *N. Sanderae*

A discussion of this cross would not be complete without first reviewing an article of recent date dealing with *N. Sanderae*. SACHS-SKALINSKA (1921) has studied the inheritance of flower color in a species cross between *N. Langsdorffii* and *N. Sanderae*. She found eight factors influencing

flower color in *N. Sanderae*. The relation of these factors may be summarized as follows:

F is a basic factor necessary for the production of anthocyanin pigment in the cell sap. The cell sap is violet in presence of *F* alone.

C acting in the presence of *F* produces a red color in the cell sap. This change is not complete, as microscopic studies of red corollas show a limited number of cells filled with violet pigment.

The relation of these two factors may be further illustrated thus:

FC = red corolla	*fC* = white corolla
Fc = violet corolla	*fc* = white corolla

I_1 and I_2 are factors determining the intensity of the red and violet colors. If only i_1 and i_2 are present the flowers will have a pale rose tinge in place of red, and lilac in place of violet. The presence of a single factor (either I_1 or I_2) slightly increases the intensity of the color. The simultaneous presence of I_1 and I_2 in the zygote distinctly increases the intensity of the flower color. These factors are quantitative in nature, the intensity of flower color depending upon the number of dominant genes present.

N and *T* are responsible for the distribution of anthocyanin in the exterior of the corolla.

N determines the appearance of a red pigment on the exterior face of the corolla with traces of red on the exterior of the tube of the corolla.

T acts only if *N* is present. In the presence of *N* it determines a very light pigmentation on the exterior tube of the corolla.

P and *R* act only if *F* or *FC* are present. They are responsible for the distribution of anthocyanin in the interior of the corolla.

P in the presence of *F* or *FC*, distributes the violet or red pigment in the cells of the epidermis of the interior of the corolla.

R in the presence of *F* or *FC*, distributes the violet or red pigment in the parenchyma of the interior of the corolla.

If both *P* and *R* are absent when *F* or *FC* are present, only traces of pigment are found in the interior face of the corolla. If both *P* and *R* are present together with *F* or *FC* the pigments are found in both the epidermis and parenchyma. In this case, the color may show through to the exterior face where the tissue is thin.

The action of these factors may be illustrated further thus:

FP
FR
FPR } interior of corolla is of a lilac color

FPI_1 FPI_2
FRI_1 or FRI_2 } interior of corolla is of a darker lilac color
$FPRI_1$ $FPRI_2$

FPI_1I_2
FRI_1I_2 } interior of corolla is of a dark violet color
$FPRI_1I_2$

The green color of *N. Langsdorffii* was found to be due to grains of chlorophyll in the cells of the parenchyma. The following factors were thought to be concerned:

Z_1 and Z_2 are factors determining corolla color, which behave as follows:

Z_1z_2 corollas are very dark green, approaching that of *N. Langsdorffii*.

z_1Z_2 corollas have a very clear cream tinge, due to the presence of a limited number of chlorophyll grains.

The factors Z_1 and Z_2 are thought to be linked according to a gametic ratio of 3 : 1 : 1 : 3 or 5 : 1 : 1 : 5.

Z_1 Further studies lead to the conclusion that this supposed factor is in reality two factors, namely *E* and *W*.

E is a factor causing the presence of chlorophyll grains, hence, green color, on the exterior of the corolla.

W is a factor which in the presence of *E* causes grains of chlorophyll in the interior of the corolla. *Ew* produces a green exterior and a white interior corolla color.

The two series of factors introduced by the two species are transmitted independently and both series can be present simultaneously in the zygote. Hence, it is possible to unite grains of chlorophyll with a cell-sap pigment in the same tissue and cells. The F_1 plants had red and violet anthocyanin pigment in their tissues, and in some cells, grains of chlorophyll were found along with the pigments. In the second generation, obtained by self-fertilization, a very complex segregation was found. In the second and third generations, new types were found besides those corresponding to the parent plants. The complete article has not been available, so that we have not been able to examine the data critically, but we have included the foregoing discussion for what bearing it may have on our investigation.

In our investigation, three crosses were made between *N. longiflora* and *N. Sanderae* as follows:

23 F_1 24 = *N. longiflora* (22-35 P−1) × *N. Sanderae* (22-53 P−1)
23 F_1 25 = *N. longiflora* (22-35 P−11) × *N. Sanderae* (22-53 P−1)
23 F_1 26 = *N. longiflora* (22-35 P−11) × *N. Sanderae* (22-53 P−3)

These three hybrids were all alike in all the vegetative characters. *N. Sanderae* is also fairly uniform in this respect. The differences which do occur are concerned with the size of the rosette in the early stage of growth, the vigor of the plant in later stage of growth, and the color of the midrib. The color of the midrib varies in different plants in the rosette stage from a light yellow to a deep purple color, but the purple color seems to fade in the later stages of growth. These differences were not studied in detail. The characteristics of the parents and hybrids are presented in table 2. Observations on flower color and size are presented in a subsequent paragraph.

TABLE 2

Comparison between the parents and F_1 hybrid between N. longiflora and N. Sanderae.

N. longiflora	$23F_1$ 24, $23F_1$ 25, $23F_1$ 26	*N. Sanderae*
Stems slender and bristly -scabrous	Stems viscid-pubescent and stouter than *N. longiflora*	Stems viscid-pubescent and stouter than *N. longiflora*
Bushy habit of growth, but less than *Sanderae*	Like *Sanderae* and more vigorous than either parent	Bushy habit of growth
3–4 feet in height	4–4½ feet high	3–4 feet in height
Leaves spatulate to lanceolate, distinctly undulate, obtuse or acute; auricles extend half-way around the stem	Auricles extend half-way around the stem; otherwise like *Sanderae*	Rosette leaves large, spatulate, distinctly undulate. Caulescent leaves oblong-lanceolate, acuminate, undulate. A narrow strip of leaf blade extends a short distance down the stem, beyond the union of leaf and stem
Corolla yellowish-violet without, white within	Corolla color various	Corolla color various, see subsequent discussion
Corolla, salverform, slightly swollen above	Like *N. Sanderae*	Corolla salverform, decidedly swollen above; about twice as stout as *N. longiflora*
Stamens adnate to corolla their entire length	Like *Sanderae* except there is no bulge inward where the stamen changes from the adnate to the free condition	Stamens adnate to corolla for about ¾ of their length, being free 1 inch from apex downward. A distinct bulge inward where the stamen changes from the adnate to the free condition
Flowers night-opening	Flowers night-opening	Flowers open day and night

Two plants of *N. longiflora* grown in 1922 and fifty-five plants grown in 1923 were all of a uniform type. Our sample of *N. Sanderae* was obtained from THE ROYAL BOTANIC GARDENS, Kew, England. *N. Sanderae* was originated in 1903 by SANDER AND SONS, St. Albans, England, as a cross between *N. alata* var. *grandiflora* and *N. Forgetiana* (HEMSLEY 1905, RICKER 1916). *N. alata* has white interior corolla lobes, which are pale violet on the exterior. The corolla of *N. Forgetiana* is rose-colored. RICKER (1916) describes *N. Sanderae* as having a rose-colored corolla. SACHS-SKALINSKA (1921) has referred to it as being red and having a few cells containing a violet pigment. SETCHELL (1912) has stated that it

"has exhibited a considerable variety of form and color of flower and some variability in habit and fertility. All the true *N. Sanderae* show the influence of the red-flowered parent (*N. Forgetiana*) not only in color, but also in the strongly developed gibbous ring in the throat of the corolla just below the limb."

EAST and PARK (1917) were the first to study in detail the sterility factors in the hybrids of *N. Sanderae* and *N. alata*. We have found *N. Sanderae* to be self-sterile when enclosed in paper bags, but the plants set open-pollinated seed in the garden. Our sample had no history except that it was *N. Sanderae*. Our observations show that it is composed of several different colors. The following scheme of classification for the different shades of corolla color was adopted for the 1923 studies:

Color No. 1. Interior of corolla lobes white when first opened, but the veins become Pale Lobelia Violet[2] on exposure to the sun. Exterior face of corolla lobes and tube an Oil Yellow blended with Vinaceous Purple, before the flower opens. The Vinaceous Purple changes to Daphne Red as the flower matures.

Color No. 2. The same as color No. 1 except the interior and exterior face of the corolla lobes becomes Light Lobelia Violet over nearly the entire surface on exposure to the sun.

Color No. 3. Interior face of corolla lobes Chinese Violet when first opened, deepening to Rood's Violet on exposure to the sun. Exterior face of corolla lobes and corolla tube an Oil Yellow, the lobes washed with Corinthian Purple and the tube with Rood's Violet.

Color No. 4. Same as color No. 3 except that on exposure to the sun the interior face becomes entire Rood's Violet with Pansy Violet veins.

Color No. 5. The same as color No. 1 except that the exterior face of the corolla lobes and tube are washed with Mulberry Purple.

Color No. 6. Interior face of corolla lobes Magenta, deepening to Dull Magenta Purple on exposure to the sun. Exterior face of corolla lobes Dull Dark Purple and the corolla tube Rood's Violet.

[2] Color terminology from RIDGWAY (1912).

Color No. 7. Interior face of corolla lobes Mauvette with veins of Chinese Violet. Exterior face of corolla lobes Corinthian Purple to Rood's Violet.

Color No. 8. Interior face of corolla lobes Rhodamine Purple. Exterior face of corolla lobes Pomegranate Purple. Exterior color of corolla tube Rhodamine Purple.

Color No. 9. Interior face of corolla lobes a Mallow Pink with veins of Tyrian Pink. Exterior face of corolla lobes an English Red, and the exterior tube Rhodamine Purple.

These colors may be arranged into four groups according to their general similarities. The members of these four groups differ from each other by slight shades of color:

(a) the sun-lilac group, colors 1, 2, 5;
(b) the violet group, colors 3, 4, 6;
(c) the light-violet group, color 7;
(d) the red group, colors 8, 9.

Three plants of *N. Sanderae* were grown in the spring of 1922. The three plants all seemed to differ from one another, as their description will indicate. The red group was not represented in the 1922 planting, but was found in 1923. The color descriptions made from RIDGWAY'S "Color standards" furnish a fairly accurate comparison of the plants grown during a previous year. It is always desirable, however, to raise the parents from self-fertilized seed the following year for comparison, or to propagate them asexually in case they are self-sterile.

Description of the plants of *N. Sanderae* grown in 1922

Plant No. 1. The interior of the corolla was Mauvette, with veins of Chinese Violet. Probably equivalent to color No. 7.

Plant No. 2. The interior of the corolla lobes was a Chinese Violet, with veins of Rood's Violet. Equivalent to colors 3 or 4.

Plant No. 3. The interior of the corolla lobes was a Pale Lobelia Violet with veins of Chinese Violet. Equivalent to color No. 2.

The 1923 planting was sown from the same original package, since no self-fertilized seed could be obtained in 1922. As a matter of fact, not even open-pollinated seed set on *N. Sanderae* in the greenhouse at Urbana, Illinois, and it was impracticable to propagate them asexually and transport them to California. The distribution of flower color for the 1923 population will be found in table 3. The number of plants in each group is of no immediate significance in the inheritance of flower color, since we do not know the history of the sample. In table 3 we have shown the segre-

gation for flower color for the F_1 hybrids and the average spread and length of corolla for plants of the same flower color. Concerning the intensity of interior corolla color, we may group colors 1, 2 and 5 together as the sun-lilac group, and colors 3, 4 and 6 together as the violet group. This is justifiable, for the interior corolla colors of the sun-lilac group were practically alike, and colors 3, 4 and 6 were separated only by shades. The sun-lilac group can be accurately separated from the violet group by the absence of lilac before the flowers open.

In treating the data in this manner we obtain a 1 : 1 ratio of sun-lilac to violet in all three hybrid populations. This is the type of ratio we expect from a back-cross. If the factor scheme proposed by SACHS-SKALINSKA is accepted, it is evident that we are dealing with an intensity factor or possibly one of the factors responsible for the distribution of anthocyanin in the interior of the corolla. In the summary which was available for our use, however, no mention was made of the development of color upon exposure to the sun. In collecting the data, two unopened, two fully opened, and two mature flowers were collected from each plant. The flowers from each plant were then arranged into groups according to color, and these groups were then described as they happened to come. Hence, there was very little chance of conscious selection. From an inspection of table 2, it is evident that the majority of characters of *N. Sanderae* are dominant over those of *N. longiflora*. It would also seem that the flower color of *N. Sanderae* is dominant in the F_1 hybrids as far as interior corolla color is concerned. If we are correct in this assumption, we are dealing with the equivalent of a back-cross. *N. Sanderae* is heterozygous in its genetic constitution (for flower color, etc.) and hence may be analyzed by crossing with *N. longiflora*. The data with regard to exterior shades does not warrant any conclusion as to genetic differences.

A critical observer could notice from a field inspection that there was a difference in flower size in *N. Sanderae* and in the F_1 hybrids. Since *N. alata* and *N. Forgetiana* differ considerably in flower size, *N. Sanderae* may be heterozygous for flower size. Since *N. longiflora* is very uniform, the differences which were found in the hybrids are probably due to *N. Sanderae*. In *N. longiflora*, the corolla spread averages about 4 cm, and the corolla length about 10 cm. Detailed measurements of *N. Sanderae* in 1922 and of *N. longiflora* in 1923 were not made, although we recognize the desirability of making them.

We have listed the average length and spread of the corolla in centimeters for the plants of a given flower color in table 3. The average for color 3 is lower than for the other colors in *N. Sanderae*. The average

for color 5 is decidedly lower than the other colors in 23 F_1 25, and the same tendency is seen in 23 F_1 24. Color No. 3 is the low group in 23 F_1 26. That there is a hereditary difference in flower size is indicated by the higher

TABLE 3

Corolla color and average flower size in centimeters, of plants of a given color in N. Sanderae and F_1 species hybrids.

CULTURE NUMBER	TYPE OF OBSERVATION	COLOR NUMBER									TOTALS AND AVERAGES
		1	2	5	7	3	4	6	8	9	
22–53			Plant 3		Plant 1	Plant	No. 2				
23–53	Number of plants				7	11			2	4	24
	Corolla spread				6.5	5.9			6.8	6.5	
	Corolla length				10.3	9.4			10.1	10.0	
23F$_1$ 24	Number of plants	10		11		17	7				45
	Corolla spread	5.5		4.7		5.3	5.0				5.2
	Corolla length	9.7		9.1		9.0	9.3				9.2
23F$_1$ 25	Number of plants	39	8	9		39	9	11			115
	Corolla spread	5.2	5.2	4.3		5.2	5.1	5.0			5.1
	Corolla length	9.7	9.3	8.4		9.5	9.6	9.4			9.4
23F$_1$ 26	Number of plants	26	5			22	8				61
	Corolla spread	6.1	5.6			6.1	6.3				6.1
	Corolla length	10.0	9.0			10.3	10.2				10.1

Summary of distribution of corolla color and average flower size of plants of a given color type in the F_1 species hybrids.

CULTURE NUMBER	COLORS 1, 2, 5, THE PALE SHADES			COLORS 3, 4, 6, THE DARK SHADES		
	Number of plants	Corolla spread	Corolla length	Number of plants	Corolla spread	Corolla length
$23F_1$ 24	21	5.1	9.4	24	5.2	9.1
$23F_1$ 25	56	5.1	9.4	59	5.1	9.5
$23F_1$ 26	31	6.0	9.9	30	6.2	10.3

TABLE 4

Frequency distribution for spread of corolla.

CULTURE NUMBER	COROLLA SPREAD IN CENTIMETERS	COLOR NUMBER									TOTAL FOR THE POPULATION
		1	2	5	7	3	4	6	8	9	
23–53	4.6–5.0					1					1
	5.1–5.5				1	1					2
	5.6–6.0				1	5					6
	6.1–6.5				1	3			1	2	7
	6.6–7.0				3	1				2	6
	7.1–7.5								1		1
	7.6–8.0				1						1
Total					7	11			2	4	24
$23F_1$ 24	3.1–3.5			1							1
	3.6–4.0					1					1
	4.1–4.5			4			2				6
	4.6–5.0	1		2		5	2				10
	5.1–5.5	6		3		6	3				18
	5.6–6.0	2		1		4					7
	6.1–6.5	1				1					2
Total		10		11		17	7				45

TABLE 4 (continued)

CULTURE NUMBER	COROLLA SPREAD IN CENTIMETERS	COLOR NUMBER									TOTAL FOR THE POPULATION
		1	2	5	7	3	4	6	8	9	
	3.6–4.0			5				1			6
	4.1–4.5	6				3	1	1			11
23F$_1$ 25	4.6–5.0	11	3	4		10	3	3			34
	5.1–5.5	12	4			22	5	6			49
	5.6–6.0	8				2					10
	6.1–6.5	2	1			2					5
Total		39	8	9		39	9	11			115
	5.1–5.5	3	3			4					10
	5.6–6.0	10	2			7	3				22
23F$_1$ 26	6.1–6.5	11				7	3				21
	6.6–7.0					4	1				5
	7.1–7.5	2					1				3
Total		26	5			22	8				61

averages of 23 F_1 26 as compared with 23 F_1 24 and 23 F_1 25. The last two populations had the same *Sanderae* parent (22-53 P_1), while 22-53 P-3 was used as the female parent of 23 F_1 26. That this difference between populations is not due to mere chance is shown by the frequency distributions for spread of corolla in table 4. The range for 23 F_1 24 and 23 F_1 25 begins further to the left side of the abscissa and does not extend as far to the right as 23 F_1 26. Likewise, the ranges for flower size of plants of the same color within a given population, mentioned above, indicates that there may be hereditary differences associated with flower color. Due to the smaller number of individuals in these distributions, the evidence is less conclusive. The fact that the population as a whole exhibits a normal frequency distribution rather than a bimodal curve also renders the supposition somewhat improbable. When we use a smaller class range for the frequency table of 23 F_1 25, there is some suggestion of a bimodal curve. However, if a large number of factors are

involved, there is the possibility of obtaining the normal frequency curve. The inheritance of flower size, however, is a matter for further study.

The main features of the cross between *N. longiflora* and *N. Sanderae* may be stated as follows:

(1) The F_1 species hybrid resembles *N. Sanderae*, with a few *longiflora* characteristics.

(2) The hybrids slightly exceed both parents in vigor.

(3) *N. Sanderae* is heterozygous for flower color, size of flower, and other less obvious characters.

(4) The *Sanderae* plants used in our crosses were heterozygous for sun-lilac and violet corolla colors, and *N. longiflora* appears to act as a recessive, thus giving in the F_1 species hybrids the gametic constitution of the *Sanderae* parent.

(5) Heritable differences in flower size are found in *N. Sanderae*. The flower size of *N. Sanderae* appears to be dominant over the flower size of *N. longiflora*.

Nicotiana Tabacum var. havanensis × *N. glauca*

In 1920, a study was made of a teratological character found in *N. Tabacum* var. *havanensis*. This character consisted in a reduced number of segments in the floral whorls. The most common condition was a tetramerous flower instead of the normal pentamerous flower of the genus. The expression of this anomaly is variable, as many gradations occur between the five-parted and four-parted types. To save space in describing the flowers, the number of floral parts to each whorl was represented by the proper Arabic numeral. The corolla was listed first, then the calyx, and finally the stamens. The numbers were separated by minus signs (−) except where the plus sign (+) was used. The plus sign was used to denote a fractional segment. Thus, a flower might have four normal lobes, with a very much reduced lobe between any two normal lobes, giving two apices very close together. This fractional segment was variable in shape, but always clearly indicated that it was an imperfectly formed lobe. Some of the types were as follows:

$4-4-4$, $5-4-5$, $4-5-4$, $5-5-4$, $5-4-4$, $4-4-5$, $4-3-4$, $4-5-5$, $5-3-4$, $3-3-3$, $5-3-5$, $4-4-3$, $4-3-3$, $3-4-3$, $4-3-3$, $5-5-3$, $5-3-4$, $4^{+}4-5$, $5-4^{+}4$, $5-4^{+}5$, $4^{+}5-5$, $4^{+}5-4$, $4^{+}4-4$, $4^{+}4^{+}4$, $5-4^{++}5$, $4^{+}4^{+}5$, $4-4^{+}4$, $4-4^{+}5$, $3^{+}4-3$, $5-3^{+}4$, $4^{+}5-5$, $4^{+}3-4$, $5-3^{+}5$, $3^{++}3-3$, $4^{++}4-4$, $5^{+}4^{+}3$, $4-3^{++}4$, $5-3^{++}5$, $4^{+}3^{++}4$, $3^{+}3-3$, $4-3^{+}4$, $4^{+}4^{+}3$, $4^{+}4-3$, $4^{++}5-4$, $4-6-4$, $6-5-4$, $4-7-4$, $4^{+}6-6$, $4^{++}6-5$, $4^{+}6-4$, $4^{+}6-5$, $5^{+}4-4$, $4^{+}5^{+}4$.

Not all of these types were found on *havanensis*. No floral whorls having an increased number of parts were found on *havanensis*. The other types were found on hybrids involving *havanensis*. The increased number of lobes came from var. *macrophylla* or var. *purpurea*, depending on the particular cross involved. These two varieties, particularly *macrophylla*, produce a few flowers having an increased number of floral parts. In treating the data, all of the flowers showing a reduced number of parts were added together to obtain the total number of reduced flowers. This was justified, since the data at hand did not indicate a separate inheritance for the different floral whorls.

The flowers of *N. Tabacum* remain attached to the plant for several days, and the fertilized capsule remains attached after the corolla has been shed. Records were taken often to be sure of counting all of the flowers during the flowering season until the frost came. In recording the data, all the

TABLE 5

Periodicity in appearance of reduced flowers in 19 F_1 H 182 P–46.

PERIOD NUMBER	DATE OF COUNT	TOTAL NUMBER OF FLOWERS	NUMBER OF 5–5–5	NUMBER OF 4–4–4	TOTAL NUMBER OF REDUCED FLOWERS	PERCENTAGE OF 4–4–4	TOTAL PERCENT OF REDUCED FLOWERS
1	10– 6–19	32	0	32	32	100.0	100.0
	10– 9–19	15	8	6	7	40.0	46.6
	10–19–19	60	28	17	32	28.3	53.3
Totals for 1st period		107	36	55	71	51.4	66.3
2	10–23–19	39	30	4	9	10.2	23.0
	10–25–19	15	10	4	5	26.6	33.3
	10–28–19	32	20	9	12	28.1	37.5
	11– 1–19	33	21	7	12	21.2	36.3
Totals for 2nd period		119	81	24	38	20.1	31.9
3	11– 7–19	65	50	5	15	7.6	23.0
	11–10–19	46	40	3	6	6.5	13.0
Totals for 3rd period		111	90	8	21	7.2	19.0
4	11–17–19	58	50	5	8	8.6	13.7
	11–19–19	24	20	3	4	12.5	16.6
	11–21–19	23	24	2	3	7.4	11.1
	11–24–19	40	31	1	9	2.5	22.5
Totals for 4th period		145	125	11	24	7.5	16.5

TABLE 6

Observations on the inheritance of reduced flowers.

VARIETY OR CROSS	MEAN PERCENTAGE OF REDUCED FLOWERS	RANGE OF THE PERCENTAGE OF REDUCED FLOWERS	CHARACTER OF THE FREQUENCY DISTRIBUTION
N. Tabacum var. *havanensis*	18.814 ±0.203	14.0–23.0	The frequency polygon approximated the normal frequency polygon
N. Tabacum var. *macrophylla*	0.405 ±0.013	0.0– 1.0	An asymmetrical polygon, the mode being at 0.0 percent and the polygon descending abruptly to the right
N. Tabacum var. *havanensis* × *N. Tabacum* var. *macrophylla* Culture No.= 19 F_1 H 174	0.688 ±0.756	0.0– 4.0	An asymmetrical polygon, the mode being at 0.0 percent. The polygon descends abruptly to the right
19 F_1 H 174 × *N. Tabacum* var. *havanensis*	———	0.0–46.0	An asymmetrical polygon, the majority of the plants being found between 0.0 and 12.0 percent, the mode of the "first smooth" being found at 0.0 percent. The polygon descends gradually toward the right
N. Tabacum var. *purpurea*	———	———	None observed
F_1 *N. Tabacum* var. *havanensis* × *N. Tabacum* var. *purpurea*	1.127 ±0.792	0.0– 6.0	An asymmetrical polygon, the mode being at 1 percent, the polygon descending abruptly to the right
F_2 *N. Tabacum* var. *havanensis* × *N. Tabacum* var. *purpurea* and reciprocal	———	0.0–54.0	An asymmetrical polygon of the J-type, the mode of the "first smooth" being found at 0.0 percent

expanded flowers were removed from the plant, the number of floral parts to each whorl being recorded as the flowers were removed from the plant. As soon as the individual flowers were recorded, they were discarded, so that there would be no possibility of recording the same flower twice.

A study was made of the periodicity in the appearance of abnormal flowers on one plant of a sesqui-hybrid between (*N. Tabacum* var. *havanensis*×*N. Tabacum* var. *macrophylla*)×*N. Tabacum* var. *havanensis*. We have reproduced table A from MALLOCH (1920) and have renumbered it table 5. The data were grouped into four periods arbitrarily, so that the total for each group would approximate one-hundred flowers. The percentage for these four groups show a steady decrease, while the percentages for the individual dates show a fluctuating decrease downward. *N. Tabacum* var. *havanensis* shows this same periodicity in the appearance of reduced flowers. Since the results of the 1920 studies are not generally available, we have also prepared a brief summary (table 6) of the observations on the tetramerous character, in order that the present investigation may be better understood.

It was planned to continue this problem and to study the inheritance of reduced flowers in species crosses. The *N. glauca* planting at Urbana did not come into bloom early enough to be utilized for crossing. Through the kindness of Mr. M. A. KELANEY, we were given seeds of a cross between *N. Tabacum* var. *havanensis* and *N. glauca*, which he had made for another purpose, and hence, we have been able to observe the behavior of reduced flowers in this cross. The F_1 plants were found by the writers to be self-sterile. Mr. KELANEY also found them to be self-sterile. The tests on back-crosses to *havanensis* have not been completed. EAST and HAYES (1912) found that the F_1 hybrids of reciprocal crosses of *N. Tabacum* and *N. glauca* were sterile. Since this cross is the subject of another investigator, we shall confine our discussion largely to the inheritance of reduced flowers. Suffice it to say that the plants produced a considerable number of flowers, thus facilitating observations on this character. The flowers more nearly resembled *N. Tabacum* var. *havanensis* than *N. glauca* in shape and size, which was also an aid in comparing the F_1 hybrid and the female parent.

Our observations on the hybrid between *N. Tabacum* var. *havanensis* and *N. glauca* could not be as extended as the studies in 1920. Observations were made in the beginning of the blooming period extending over a period of two weeks, and observations were again made toward the close of the blooming period. Since it was found in the earlier studies that all of the reduced flowers could be treated together, this practice was followed in this study. In the earlier investigation, an equal number of flowers were counted on all the plants used in the calculations. This has not been done in our present study, due to lack of time. The necessity for this technique is partly mathematical and partly physiological. It is, of course, necessary

to secure a sufficiently large sample to contain a fair representation of the possible percentages of the different types of flowers produced. The cause of the periodicity in the appearance of reduced flowers, whether due to the age of the plant, or to the season of the year, etc., would also influence the kind of records to be kept. In this study, observations were made on all of the plants and on all of the flowers on the same day. Hybrids 23 F_1 156 and 23 F_1 157 are both crosses between *N. Tabacum* var. *havanensis* and *N. glauca:* 23 F_1 156 = *N. Tabacum* var. *havanensis* (22.040 P-10) × *N. glauca* (22.024 P-X); 23 F_1 157 = *N. Tabacum* var. *havanensis* (22.040 P-9) × *N. glauca* (22.024 P-X)

The parents of these hybrids have been inbred for a number of years at the UNIVERSITY OF CALIFORNIA. The plants of 23 F_1 156 came into flower at different dates; therefore, the number of flowers on some plants is too small to be significant. The data for 23 F_1 157 is more uniform than 23 F_1 156 and conforms to a normal frequency distribution, similar to that of *N. Tabacum* var. *havanensis*.

TABLE 7

Frequency distribution of reduced flowers.

PERCENTAGE OF REDUCED FLOWERS	23 F_1 157 EXCEPT PLANT 9	23 F_1 157 EXCEPT PLANT 9 23 F_1 156 PLANTS 1, 4, 5, 8, 9, 15, 16, 19-24
	NUMBER OF PLANTS	NUMBER OF PLANTS
5.1–15.0	1	1
15.1–25.0	3	3
25.1–35.0	3	7
35.1–45.0	6	10
45.1–55.0	7	11
55.1–65.0	3	4
65.1–75.0	2	2
Total	27	40

The data for 23 F_1 156 (table 8) indicates that there is a decrease in the percentage of reduced flowers in the late blooming period. If we construct a combined frequency table for 23 F_1 156 and 23 F_1 157, including all the plants where at least twenty flowers were observed in the early flowering period, we also obtain a normal frequency distribution. This is expected, since the parents are relatively homozygous and the two hybrids exhibit about the same range and the same type of frequency distribution. A summary of the observations on this species hybrid may be enumerated as follows:

(1) There is a periodicity in the appearance of reduced flowers in the species hybrid very similar to that of *N. Tabacum* var. *havanensis*.

(2) Field observations indicated that the same general types of reduction were found in the species hybrid as in *havanensis*. No flowers were found (except two very much distorted freaks) in which there was an increased number of floral parts.

(3) Both of the hybrid populations exhibit a normal frequency distribution for percentage of reduced flowers, thus resembling *havanensis*.

(4) The teratological character, namely, reduced floral whorls, found in *N. Tabacum* var. *havanensis*, is dominant in the species hybrid over the normal pentamerous condition.

(5) Sterility of the F_1 species hybrid limits the further study of this character in this particular cross.

TABLE 8

Reduced flowers found on F_1 N. Tabacum var. havanensis×N. glauca.

PLANT NUMBER	23 F_2 156						23 F_1 157	
	EARLY PERIOD		LATE PERIOD		TOTAL		EARLY PERIOD	
	Number of flowers counted	Percent of reduced flowers	Number of flowers counted	Percent of reduced flowers	Number of flowers counted	Percent of reduced flowers	Number of flowers counted	Percent of reduced flowers
1	41	43.9	41	14.6	82	29.3	31	45.2
2	12	58.3	10	0.0	22	31.8	41	41.5
3	2	50.0	29	3.5	31	6.5	41	51.2
4	39	51.3	25	16.0	64	37.5	42	66.7
5	34	38.2	21	4.8	55	25.5	22	59.1
6	—	——	26	23.1	26	23.1	37	32.4
7	17	11.8	8	12.5	25	12.0	51	45.1
8	58	29.3	47	14.9	105	22.9	23	34.8
9	28	53.6	22	9.1	50	34.0	5	80.0
10	16	56.2	36	30.6	52	38.5	31	71.0
11	—	——	77	35.1	77	35.1	41	41.5
12	9	55.5	6	0.0	15	33.3	83	20.5
13	5	40.0	13	15.4	18	22.2	17	47.1
14	11	18.8	3	0.0	14	14.3	31	48.4
15	38	50.0	21	9.5	59	35.6	33	42.4
16	44	31.8	3	0.0	47	29.8	39	43.6
17	5	60.0	29	34.5	34	38.2	48	22.9
18	7	14.3	27	18.5	34	17.6	62	35.5
19	31	25.8	12	8.3	43	20.9	48	14.6
20	32	56.2	33	9.1	65	32.3	58	24.1
21	48	45.8	32	3.1	80	28.7	33	51.5
22	62	38.7	28	10.7	90	30.0	57	61.4
23	51	39.2	—	——	51	39.2	56	28.6
24	76	28.9	11	0.0	87	25.3	21	47.6
25	—	——	—	——	—	——	27	59.3
26	—	——	—	——	—	——	37	40.5

DISCUSSION OF RESULTS

The crosses enumerated in this article afford examples of varying degrees of fertility in species hybrids in Nicotiana. Add to this the results discussed by other investigators and we have quite a complete range of fertility in the species hybrids of this genus. Our search for hybrids which might parallel the *N. Tabacum*×*N. sylvestris* cross has in part confirmed the work of EAST and HAYES (1912) on the degree of fertility exhibited by various species crosses. The instances where our results have failed to agree with those of previous investigators have been listed in the beginning of the paper. These cases have been sufficient to indicate that environmental conditions and perhaps varietal or strain differences greatly influence the success of a cross. Environmental conditions might include lack of optimum conditions at the time of seed development and the method of germination. The hybrid between *N. longiflora* and *N. Tabacum* is an excellent illustration of the influence environmental conditions or the variety may have on the success of a species cross. By referring to table 1 it will be seen that several different varieties of *N. Tabacum* were used with *N. longiflora* as one parent. The seeds from the cross of *N. Tabacum* var. "Connecticut Broadleaf" with *N. longiflora* failed to germinate. One plant which reached maturity was obtained from *N. longiflora* ×*N. Tabacum* var. "Little Dutch," while the seedlings of another cross involving different plants of the same parents died when the food material in the seed was exhausted. A more obvious difference is found in Mr. KELANEY'S cultures, which demonstrate marked differences in *N. Tabacum*×*N. glauca* crosses depending upon the particular variety used in the cross.

All of the crosses which we have listed set seed, but some of those which failed to germinate appeared to consist of empty capsules. In the case of the crosses producing empty shells, the ovules may become abortive after fertilization has taken place. Seeds which appear to be morphologically perfect may fail to germinate if the reaction systems are sufficiently dissimilar. The data which has been collected by investigators is not sufficient at present to warrant the statement of a general law regarding the influence the chromosome number has upon the vigor and fertility of species crosses. For example the cross of *N. Bigelovii* ($n=24$)×*N. longiflora* ($n=10$) involves the same number of chromosomes as the cross *N. Tabacum* ($n=24$)×*N. longiflora*, but the latter is by far the more vigorous hybrid. A parallel example is found in the hybrids of *N. sylvestris* ($n=12$) ×*N. Tabacum* (GOODSPEED and CLAUSEN 1917) and *N. rustica* ($n=24$)× *N. paniculata* ($n=12$) (EAST 1921), in which the latter cross was more

fertile than the former. COLLINS and MANN (1923) secured very abnormal reduction phenomena from the F_1 of *Crepis setosa* ($n=4$) $\times$ *Crepis capillaris* ($n=3$), while *C. setosa* $\times$ *C. biennis* ($n=20$) showed almost normal reduction. EAST and HAYES (1912) secured vigorous growth from the cross *N. rustica* $\times$ *N. Langsdorffii* ($n=9$). The chromosome numbers in this case are not even multiples unless 3 were taken as the basic number. It would be impossible to fit the species of Nicotiana into a multiple series without taking 1 as the basis, and such a standard would have no significance.

One fact seems fairly evident in the cytological studies of species hybrids, that in some cases at least where the hybrid shows a fair degree of compatibility, there are pairs of chromosomes having considerable likeness of internal composition. Thus, COLLINS and MANN (1923) conclude from their Crepis studies that ". . . it is evident that normality of reduction does not depend upon similarity of chromosome number, but rather upon the internal composition of the chromosomes." GOODSPEED (1923) thinks that the 12 bivalent plus 12 univalent chromosomes present in the F_1 hybrid of *N. sylvestris* $\times$ *N. Tabacum*, represent 12 *sylvestris* plus 12 *Tabacum* chromosomes and 12 unpaired *Tabacum* chromosomes. TÄCKHOLM (1922) concludes from the cytological studies presented to date on species hybrids, that when pairing occurs the maternal chromosomes tend to pair with the paternal, leaving the extra chromosomes unpaired. An exception is found in *Crepis setosa* ($n=4$) $\times$ *C. biennis* ($n=20$), where there is evidence that the *biennis* chromosomes mate with one another (COLLINS and MANN 1923). STURTEVANT (1921) has pointed out that there is a parallelism between the chromosomes of *Drosophila melanogaster* and *D. simulans*. The chromosomes are identical in shape, size and number in these two species. The loci for the genes of similar characters in the two species are located in the same approximately linear order. The two species are very similar in appearance and are only separated by minute differences. When crossed they give sterile offspring. Difference in chromosome number is eliminated in the causes of sterility in a species hybrid of this type. Whatever the correct explanation of sterility in species hybrids may be, it is apparent that most of the attempted hybrids listed in table 1 are not suitable for studies on the theory of reaction systems. Let us now turn to a consideration of the three fairly successful hybrids described in this article.

The species hybrid involving *N. longiflora* and *N. Tabacum* would only be of value as the equivalent of a back-cross for certain characters, as flower color and flower size. The fringed corolla lobes of the species hybrid

would tend to complicate such a character as reduced number of floral whorls, as found in *N. Tabacum* var. *havanensis*. The low percentage of germination of the hybrid seed would render such a cross very unreliable in representing all of the combinations of a varietal cross in *N. Tabacum*. The reduced vigor of the species hybrid also renders it unsatisfactory for garden experiments. This is an example of a species hybrid which is somewhat similar to *N. rustica*×*paniculata* except in the degree of vigor and fertility exhibited, since it resembles the parent with the high chromosome number with a few characteristics of the other parent. Another parallel case is found in the Crepis experiments already referred to, in which the F_1 hybrid of *C. setosa* ($n=4$)×*C. capillaris* ($n=3$) resembled *C. setosa* except in one character. The F_1 hybrid of *C. setosa*×*C. biennis* resembled *C. biennis* except in about two characters. Our cross of *N. longiflora*×*N. Sanderae* will illustrate, however, that dominance is not necessarily associated with the larger chromosome number. It is interesting to note that while *N. Tabacum* is nearly entirely dominant over *N. sylvestris*, it is only partially dominant over *N. longiflora*, and one of our associates has material to show that *N. Tabacum* is less dominant over another species. Thus we have a series for the degree of dominance in species hybrids involving *N. Tabacum* as one parent. In terms of the reaction-system hypothesis, our hybrid represents a case in which certain groups of characters in the *N. Tabacum* reaction system entirely dominate over those of *N. longiflora*, but in which other factors of the two parents are able to work together to produce an intermediate result. Other groups of factors in the two systems are antagonistic, resulting in reduced vigor and fertility.

It seems evident from the literature that *N. Sanderae* is a heterozygous hybrid from *N. alata* var. *grandiflora* and *N. Forgetiana*. Our sample exhibited the polymorphism for size factors and flower colors that is supposed to exist in this form, but did not represent all the types which have been found. The typical haploid number of chromosomes for *N. alata* is nine (GOODSPEED 1923), and other evidence given to us would indicate that this is also the typical number for *N. Forgetiana* and *N. Sanderae*. Due to the interfertility of these forms, some of the Nicotiana experts have come to regard them as varieties of one species. We have used the designations in common use in order to be clear as to what forms are under discussion. For a proper analysis of this cross, *N. Sanderae* should be synthesized from *N. alata* and *N. Forgetiana* and the history of the different color types accurately traced. One could then compare the second generation of this cross with the cross of the F_1 to *N. longiflora*.

If *N. longiflora* behaved as a true recessive, a valuable method of analyzing *N. Sanderae* would be established. By crossing *N. longiflora* with *N. alata* and *N. Forgetiana*, one should also be able to test the recessive nature of *N. longiflora*. Some of these crosses have been made, but our observations have not been completed. Our results seem to indicate that *N. longiflora* can be used as the equivalent of a back-cross in analyzing the color characters of *N. Sanderae*. The fairly high percentage of germination of the hybrid seed gives the species hybrid a fair chance of representing all of the recombinations of *N. Sanderae*. The vigor of the hybrids gives them practical value in hybridization studies, since they may be readily propagated.

The development of color in the corollas of some forms of *N. Sanderae* is similar to the development of plant colors in corn described by EMERSON (1921). It is another illustration of the range of character expression depending upon environmental conditions. A character of this type as well as the teratological character in *havanensis*, which fluctuates according to environmental conditions, should throw some light upon the nature of the gene. A color character which remains undeveloped, except on exposure to light, is good evidence that a chemical reaction is brought about by the action of light. These characters give some ground for believing that the genes may be of the nature of enzymes, and that like all enzymes they may have optimum and limiting factors. Such optimum conditions might account for the periodicity in appearance of reduced flowers in *havanensis*. By referring to table 5, it will be noted that the percentage of reduced flowers decreased as the season progressed, thus giving the plants less light and a lower temperature. The development of color in certain forms of *N. Sanderae* is not such a delicately balanced reaction, the development of color simply depending on a certain amount of sunlight.

The cross of *N. longiflora* ($n=10$) $\times$ *N. Sanderae* ($n=9$) is an example of a species hybrid in which dominance is not associated with the larger chromosome number. The contrast between morphological characters of these two parents is relatively small as compared with the contrast between *N. longiflora* and *N. Tabacum*. Likewise *N. sylvestris* and *N. Tabacum* show greater resemblances than *N. longiflora* and *N. Tabacum*. Vigor in species hybrids of Nicotiana is not necessarily associated with morphological resemblance, as EAST and HAYES (1912) secured vigorous growth from *N. rustica* $\times$ *N. Tabacum*, and these species differ from one another in many characters. The reaction system of *N. Sanderae* is dominant over that of *N. longiflora*, with the exception of a few characters. The

interpretation of the hybrid will depend upon further cytological studies and further breeding experiments. There is the possibility that in a hybrid of this type we should obtain 9 bivalent plus 1 univalent chromosomes in the heterotypic metaphase. If this proved to be the case the gametes produced by the F_1 hybrid would contain either nine or ten chromosomes, since one member of each nine pairs would go to opposite poles and the extra chromosome would be distributed at random to either group. If the bivalent chromosomes are distributed at random to either pole, as would be expected, we would obtain the following recombination series in the gametes of the F_1 hybrid of *N. longiflora*×*N. Sanderae*.

1 (1 L plus 0 L plus 9 S),	1 (0 L plus 9 S)
9 (1 L plus 1 L plus 8 S),	9 (1 L plus 8 S)
36 (1 L plus 2 L plus 7 S),	36 (2 L plus 7 S)
84 (1 L plus 3 L plus 6 S),	84 (3 L plus 6 S)
126 (1 L plus 4 L plus 5 S),	126 (4 L plus 5 S)
126 (1 L plus 5 L plus 4 S),	126 (5 L plus 4 S)
84 (1 L plus 6 L plus 3 S),	84 (6 L plus 3 S)
36 (1 L plus 7 L plus 2 S),	36 (7 L plus 2 S)
9 (1 L plus 8 L plus 1 S),	9 (8 L plus 1 S)
1 (1 L plus 9 L plus 0 S),	1 (9 L plus 0 S)

On the basis of this series there would be 1 chance out of 1024 of obtaining a combination of 10 *longiflora* chromosomes in one gamete, and the same would hold true for the chromosomes of *N. Sanderae*. If the plants were self-fertilized there would be 1 chance out of 1,048,576 of obtaining a homozygous parental combination. According to the theory advanced by GOODSPEED and CLAUSEN (1917), only the gametes at the extreme ends of the series are functional in partially sterile species crosses. This would account for the sterility exhibited in our species cross. But *N. Sanderae* has sterility factors which would complicate the analysis of sterility in the species hybrid. If crossing over is possible between bivalent chromosomes in a species hybrid the chances of obtaining a homozygous parental combination would be decreased again. Instead of *Sanderae* chromosomes pairing with *longiflora* chromosomes, the *Sanderae* chromosomes might pair with each other, leaving the *longiflora* chromosomes unpaired, similar to the *Crepis setosa*×*C. biennis* cross. Since the cytological studies have not been made we shall leave the discussion at this point. The many forms of *N. Sanderae* as well as different size factors in different pure lines of *N. longiflora* make this species hybrid valuable for future study.

Since the expression of "reduced flowers" in *N. Tabacum* var. *havanensis* is quantitative in nature, several factors or genes may be involved. One might assume that *havanensis* has a basic factor pair *aa* for the production of reduced flowers, along with other modifying factors. Other *N. Tabacum* varieties may possess factors which, acting with the *aa* factor, may increase the percentage of reduced flowers, as in the second generation of *N. Tabacum* var. *havanensis*×*N. Tabacum* var. *purpurea*. Since this character is quantitative in nature, a back-cross to *havanensis* would tend to increase the percentage of reduced flowers. Since "reduced flowers" is a dominant character in the species crosses with *N. glauca* and *N. sylvestris*, this type of hybrid should furnish a valuable means of analyzing F_1 hybrids between *N. Tabacum* var. *havanensis* and other varieties of *N. Tabacum*. This principle was pointed out by GOODSPEED and CLAUSEN (1917) for varietal hybrids in *N. Tabacum* when crossed with *N. sylvestris*. In the case of a character like reduced flowers, the use of the two species would serve as an admirable check, but due to the vast amount of work required in making these observations it would only be practical to use one species cross. In the species crosses, we may assume that the part of the reaction system of *N. Tabacum* var. *havanensis* concerned with the number of floral whorls dominates over those of *N. glauca*. Hence, a species cross between an F_1 and F_2 . . . F_n plant (of a *N. Tabacum* var. *havanensis*×*N. Tabacum* var. "X" cross) and *N. glauca* or *N. sylvestris* should give, on this supposition, the exact constitution of the F_1 or F_2 . . . F_n plant. Since *N. glauca* has 12 haploid chromosomes, the recombination series for gametes in the F_1 hybrid with *N. Tabacum* might be expected to be the same as the recombination series for the F_1 hybrid of *N. sylvestris*×*N. Tabacum*. This would of course depend on the nature of the reduction division. If 12 *Tabacum* chromosomes should pair with the 12 *glauca* chromosomes during the metaphase, with a random distribution of the 12 univalent *Tabacum* chromosomes during the anaphase, the recombination series for the F_1 gametes would be obtained by expanding the expression $(1+1)^{12}\times(1+1)^{12}$. This would give 169 different kinds of gametes, or only one gamete in 16,777,216 would have a chance of carrying all the chromosomes of a given parent. If only gametes containing a high percentage of parental chromosomes are functional, the sterility found in the species hybrid can be accounted for. The salient features of the investigation may be included in the following summary.

SUMMARY

(1) Seed from the following species hybrids germinated, but died in the seedling stage: *N. Bigelovii*×*N. longiflora*, *N. Bigelovii* var. *multivalvis*×*N. Sanderae*, *N. rustica*×*N. Langsdorffii*, *N. Tabacum*×*N. alata* var. *grandiflora*, *N. Tabacum*×*N. longiflora*.

(2) The success of a species cross may sometimes depend on environmental conditions, the particular variety of the species which was used, and the compatibility of the different reaction systems.

(3) The hybrid between *N. longiflora* and *N. Tabacum* var. "Little Dutch" is a weak hybrid resembling *N. Tabacum* with a few *longiflora* and a few new characteristics. This hybrid is practically sterile.

(4) The hybrid between *N. longiflora* and *N. Sanderae* is a vigorous form showing a high degree of sterility. The F_1 plants resemble *N. Sanderae* except in a few characters which show the influence of *N. longiflora*. *N. longiflora* can probably be used as the equivalent of a back-cross in analyzing color charcters in a polymorphic form like *N. Sanderae*.

(5) "Reduced flowers" is a quantitative character in *N. Tabacum* var. *havanensis*, the percentage of which can be increased by hybridization. The varietal hybrids may be analyzed by crossing them to *N. glauca* or *N. sylvestris*. If it should be possible to produce a plant having all reduced floral whorls, taxonomists could class it as a distinct species.

(6) The three successful hybrids discussed in this article are further illustrations of the effect different reaction systems have upon the development of the resulting hybrids. The sterility of the species hybrids may be explained on the basis that a high percentage of the chromosomes of a given parent are necessary to produce a functional gamete.

LITERATURE CITED

BABCOCK, E. B., 1920 Crepis—a promising genus for genetic investigations. Amer. Nat. **54**: 270–276.

COLLINS, J. L., and MANN, MARGARET C., 1923 Interspecific hybrids in Crepis. II. A preliminary report on the results of hybridizing *Crepis setosa* Hall. with *C. capillaris* (L) Wallr. and with *C. biennis* L. Genetics **8**: 212–232, *9 figs.*

EAST, E. M., 1921 A study of partial sterility in certain hybrids. Genetics **6**: 311–365, *17 figs.*

EAST, E. M., and HAYES, H. K., 1912 Heterozygosis in evolution and in plant breeding. U. S. Dept. of Agric., Bureau of Plant Industry Bull. 243. 51 pp.

EAST, E. M., and PARK, J. B., 1917 Studies on self-sterility. I. The behavior of self-sterile plants. Genetics **2**: 505–609.

EMERSON, R. A., 1921 The genetic relations of plant colors in maize. Cornell Univ. Agric. Exp. Sta. Memoir **39**: 1–156, *11 plates.*

GOODSPEED, T. H., 1923 A preliminary note on the cytology of Nicotiana species and hybrids. Svensk Bot. Tidskr. **17**[4]: 472–478.

GOODSPEED, T. H., and CLAUSEN, R. E., 1917 a The nature of F_1 species hybrids between *Nicotiana sylvestris* and varieties of *Nicotiana Tabacum*. Univ. of California Publ. Bot. **5**: 301–346, *pls. 37–48*.

1917 b Mendelian factor differences *versus* reaction-system contrasts in heredity. Amer. Nat. **51**: 31–101.

GRAY, ASA, 1876 Gamopetalae. Geol. Survey California. Vol. 1, xx+628 pp. See page 546.

HEMSLEY, W. BOTTLING, 1905 *Nicotiana Forgetiana*. Curtis's Bot. Mag. **131**: *Pl. 8006*.

LINDSTROM, E. W., 1918 Chlorophyll inheritance in maize. Cornell Univ. Agric. Exp. Sta. Memoir **13**: 1–68.

1923 Genetic research with maize. Genetica **5**: 327–356.

MALLOCH, WALTER SCOTT, 1920 Studies of inheritance in tobacco and maize. Master's thesis. 154 pp. (Manuscript) University of California Library, Berkeley, California.

1921 An F_1 species cross between *Hordeum vulgare* and *Hordeum murinum*. Amer. Nat. **55**: 281–286, *2 figs*.

RICKER, P. L., 1916 Nicotiana. Standard Cyclopedia of Horticulture. Vol. 4, pp. New York: The Macmillan Company.

RIDGWAY, R., 1912 Color standards and nomenclature. iv+44 pp. *53 pls*. Washington, D. C.: Published by the author.

SACHS-SKALINSKA, MARIE, 1921 Recherches sur les hybrides du Nicotiana. Mémoirs de l'Institut de Génétique **1921**: 110–112.

SETCHELL, WILLIAM ALBERT, 1912 Studies in Nicotiana. I. Univ. of California Publ. Bot. **5**: 1–86, *pls. 1–28*.

STURTEVANT, A. H., 1921 Genetic studies on *Drosophila simulans*. II. Sex-linked group of genes. Genetics **6**: 43–64.

TÄCKHOLM, G., 1922 Zytologische Studien über die Gattung Rosa. Acta Horti Bergiani **7**: 97–381.

YULE, G. UDNY, 1912 An introduction to the theory of statistics. xv+381 pp. London: Charles Griffen and Company.

ON MENDELIAN FACTORS IN RADISHES

J. C. TH. UPHOF
Orlando, Florida

Received January 14, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	292
Methods	293
Results	294
Long Red×Icicle	295
Early White×Early Red	296
Triumph×Early White	296
Triumph×Icicle	298
Long Red×Early White	298
Icicle×French Breakfast	300
Crosses involving Long White	301
Crosses involving Round Yellow	301
Crosses involving Black Winter	302
SUMMARY	303
LITERATURE CITED	304

INTRODUCTION

This contribution deals with some of the factors of different varieties of summer radishes, as well as the black winter radishes, relating to foliage and to color, shape and size of the roots. No observations have been made on the factors of the flowers and fruits.

I aim to analyze these characters as to their behavior in segregation and recombination and the possible occurrence of new combinations caused by compound characters.

There seems to have been a lack of extensive investigations of this highly recommendable group of varieties. The various colors, shapes, and times of development of the different varieties of radishes offer a very promising and inviting field for researches in genetics.

BARKER and COHEN (1918) worked with the varieties Scarlet Box, Scarlet Globe and White Icicle, of which the coefficients of variability were especially studied. TROUARD-RIOLLE (1920) studied the F_1 generations from reciprocal crosses between *Raphanus Raphanistrum* and *R. sativum* and found that individuals thus produced were in all cases more

vigorous than the parents. FROST (1923) found also that wild and cultivated races of radish are very heterozygous. He noticed especially self-sterility in wild races. HEGI (1921) presents an extensive description of the various forms of the radish in general.

Though the writer was able to isolate various pure lines of radish varieties which are mentioned later on, he wishes to emphasize that there is a great deal of misconception as to the names of the different varieties as sold by most seed growers. For example, the variety Triumph, obtained from several growers, could be easily distinguished in at least three different homozygous types. Though all have white roots with reddish stripes and spots, the roots of one strain were much stouter, the red on the roots darker-tinted, bearing vigorous foliage, whereas the roots of another strain, being of the same age, grown at the same time in the same soil, were smaller, more tender, the stripes lighter red and foliage smaller. A third type showed roots and foliage similar to the first-described, but the stripes were much lighter. Similar observations can be made on almost any "commercial variety"; this, however, is no hindrance in studies of variability and heredity, as soon as pure lines have been established.

METHODS

The biology of the flower has been studied sufficiently by various investigators. On account of their relatively large size they are much visited by bees and other Hymenoptera, as well as some Lepidoptera; consequently, the flowers have to be isolated carefully by paper bags. Emasculation must take place the evening before the flowers will open. There is no difficulty in handling the large flowers. As the pistil and pollen are ripe at the same time, and but few flowers of certain individuals are slightly proterogynous, they can be readily self-pollinated with a small brush, though fertilization does not occur in all ovules and therefore only a small percentage of seeds results, whereas cross-pollination has been always successful in all varieties with which the author worked. In order to be certain of having pure lines, a very large number of self-pollinations should be made and the offspring carefully studied, as not the slightest dependency can be placed upon the reliability of seed growers.

The flowers were usually pollinated in the morning, though a few were pollinated in the afternoon, but no differences could be observed in the number of seeds obtained. Great care has been taken in isolating either single flowers or entire plants.

Radish plants whose roots were studied were replanted at the desired places after they had been deprived of the leaf tops in order to prevent an excessive amount of transpiration which could not be replaced by the water supply of the root system damaged in transplanting.

RESULTS

There are many forms of radishes, which show various characteristics in height, width of foliage, nervature, earliness, and color and shape of the roots. As far as all these characteristics are concerned no new characters occurred with exception of one which was due to hybridization. For example, the shape of the roots in every homozygous strain depended upon one single factor, whether these roots were narrow, long, round or

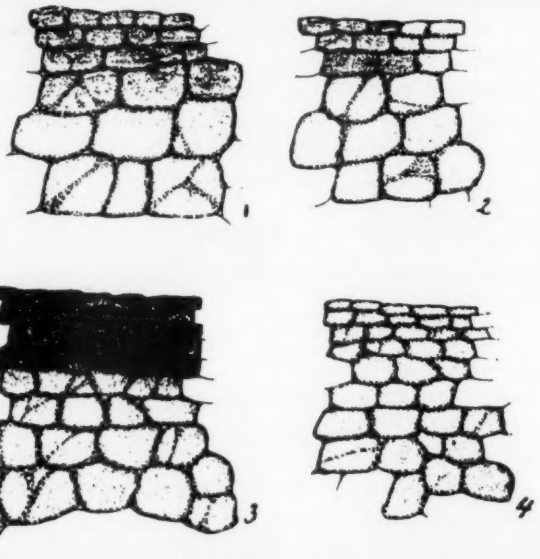

FIGURE 1.—Comparable sections of epidermis and a portion of the cortex of (1) Long Red, (2) Triumph, (3) Early Red, (4) Early White.

oval. For the study of these characters a large number of experiments was made, though they are not all mentioned in this publication. In all material with which I worked, the first hybrid generation always clearly showed intermediates with respect to size of foliage and shape of roots.

There are, however, several interesting facts concerning the color factors, especially when white and red varieties are crossed.

In red radishes the red pigment, belonging to the anthocyanin group, is to be found in the epidermis and two or three outer layers of the cortex (figure 1, 1). In the Triumph (figure 1, 2) some cells are red, and others lack anthocyanin. Figure 1, 3, is of Early Red whose red epidermis and outer cortex cells are smaller than in Long Red. Figure 1, 4, is of Early White, containing no red pigment in its roots.

The less complicated crosses will be considered first, followed by more complicated ones. In all cases reciprocal crosses were exactly alike in the F_1, and in the F_2 and F_3 the same segregations and recombinations could be noticed.

Long Red + Icicle

Of both varieties the roots are long, though in Long Red they are somewhat more delicate than in the Icicle. The shape of F_1 individuals resembles more that of the Icicle than of the Long Red. The color is entirely violet to sometimes violet-purplish, from the base to the apex of the root. Violet color of the root has been noticed in all the other crosses when white and red varieties were used.

A large number of F_1 plants, self-pollinated, produced in F_2 the results shown in table 1.

TABLE 1

YEAR	RED HOMOZYGOTE		VIOLET HETEROZYGOTE		WHITE HOMOZYGOTE	
	Number	Percent	Number	Percent	Number	Percent
1920	70	20	189	54	91	26
1921	52	26	104	52	44	22
1922	149	23	305	47	130	20

It is apparent that these color differences behave as in a monohybrid. Though in most crosses with other species or varieties of plants it is difficult or impossible to recognize the heterozygotes, it is easy to distinguish the heterozygotes between red and white radishes on account of their violet color.

Although, as stated previously, there are slight differences in shape of root between Long Red and Icicle, it is not easy to determine a proper ratio in the F_2 as to the shape of these radishes, because fluctuations in both varieties are such that boundaries between the two can not be drawn. This cross should work out as a dihybrid, and no doubt it does, although differences of shape were so difficult to observe that no attempt was made to separate the individuals definitely into homozygous and

heterozygous classes. They were noted as "behaving apparently like a monohybrid."

All the violet heterozygotes segregate in the F_3 and F_4 as they do in the F_2.

Early White × Early Red

These two varieties with short foliage and flat round roots differ from each other only in color, the former having white roots and the latter red ones with a white point.

All F_1 individuals developed violet roots with white points and short foliage. Their behavior in F_2 was like that of a monohybrid in every respect, as shown in table 2.

TABLE 2

YEAR	NUMBER OF RED ROUND	NUMBER OF HETERO-ZYGOTUS VIOLET ROUND	NUMBER OF WHITE ROUND
1921	73	148	79
1922	101	195	96

Triumph × Early White

Triumph is red-striped and has vigorous foliage, while Early White has white roots and short foliage.

Several reciprocal crosses were made between these two varieties; they all showed in the F_1 flattened roundish roots with strong to faint reddish-purple stripes and spots, on a white background. All individuals developed a strong foliage, though less vigorous than that of the Triumph (figure 2). The first hybrid generation produced, on self-fertilization, offspring with striped *versus* pure-white roots, and strong, medium or short foliage. If *R* stands for red-striped, *r* its absence, *S* for strong foliage and *s* for short foliage, and the combination *Ss* produces foliage which is practically always of medium length, there should result in the F_2, a modified di-hybrid ratio, $3RSS : 6RSs : 3\ Rss : 1\ rSS : 2\ rSs : 1\ rss$; but the following results indicate that the ratio actually present is $1\ RSS : 2\ RSs : 1\ Rss : 1\ rSS : 2\ rSs : 1\ rss$. This 1 : 1 ratio with respect to red striping suggests the action of a gamete lethal.

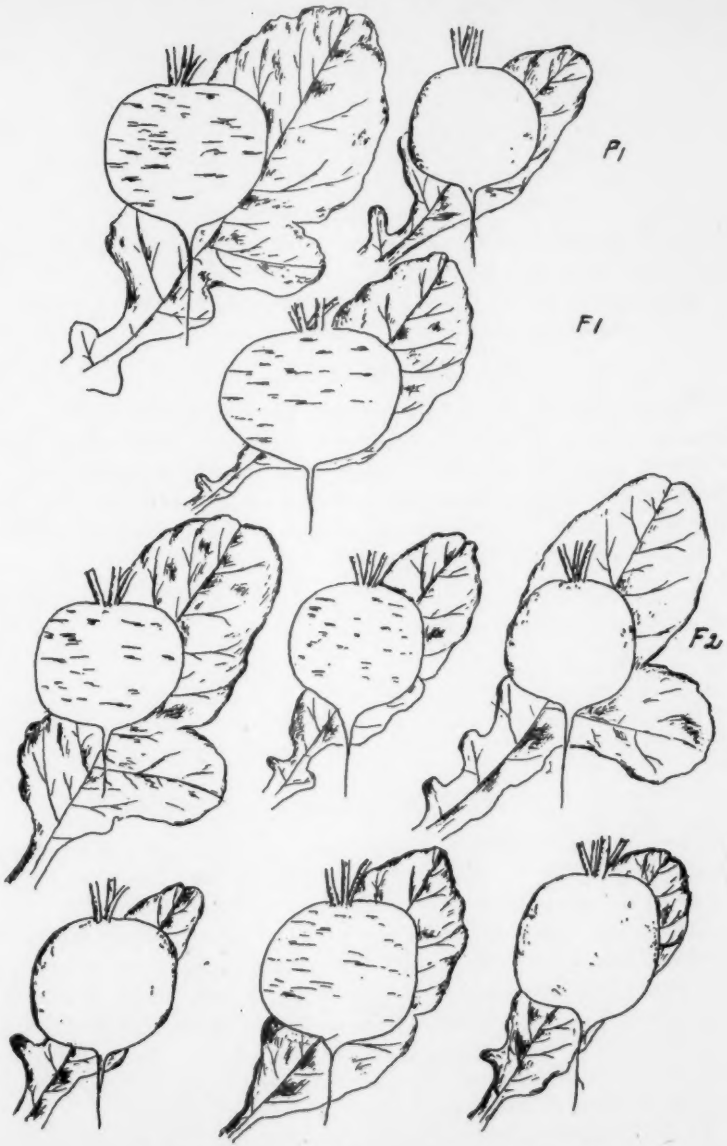

FIGURE 2.—Triumph, with vigorous foliage × Early White.

Observed	*Expected* (3 *R* : 1 *r*)	(1*R* : 1 *r*)
4	8	5
9	15	10
5	8	5
3	3	5
14	5	10
6	3	5
—	—	—
41	42	40

The fit is very close to expectation on the assumption that red-striped and white occurred in a 1 : 1 ratio.

Neither in F_1 nor F_2 was there any apparent difference between the red of a heterozygote and that of a homozygous Triumph.

Triumph × Icicle

The F_1 produces roots with a shape intermediate between the two parents, whereas the striped character is dominant. They behave like a dihybrid. It is not difficult to distinguish heterozygous individuals as far as the form of the roots is concerned (figure 3), but with respect to color only the offspring can show whether its parent was homozygous or not.

In the F_2 the following combinations were obtained:

Observed	*Expected* (1 *R* : 1 *r*)
19 with red-striped, flattened round roots	24.5
51 red-striped, intermediate in shape	49
24 red-striped, shape of Icicle	24.5
13 pure white, flattened round roots	24.5
67 pure white, intermediate	49
22 with appearance of Icicle	24.5
196	196

Long Red × Early White

The variety Long Red has long foliage, whereas Early White forms short foliage. The F_1 individuals have medium-sized foliage and broad roundish to conical-shaped roots of a violet color similar to the crosses between other white and red varieties. The second hybrid generation produced long white roots, conical white, round white, long red, conical red, round red, round violet, conical violet or long violet, with either long, medium or short foliage. None of the plants with medium foliage, conical shape or violet color of the roots bred true. The conical shape splits in round, conical and long; the violet color in red, violet and white, and the medium foliage in long, medium and short foliage.

The following combinations were obtained in this cross, with frequencies indicated:

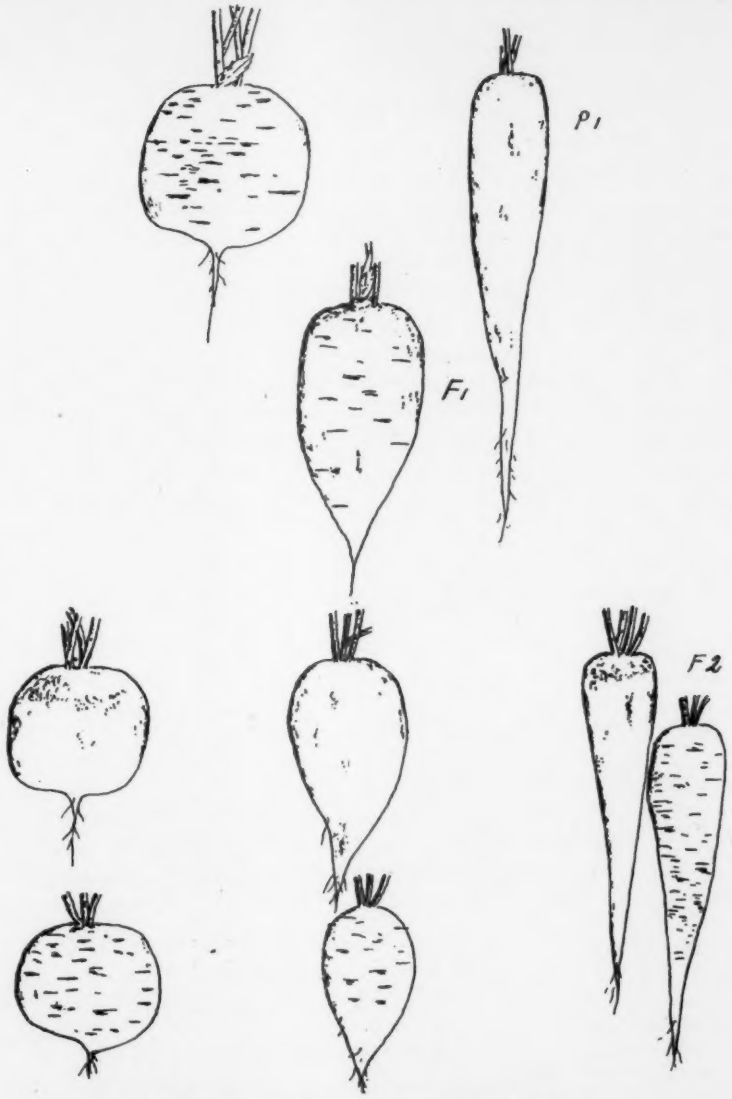

FIGURE 3.—Triumph, with vigorous foliage × Icicle.

5 red, round root, long foliage
27 red, conical root, long foliage
7 red, long root, long foliage.
9 red, round root, medium foliage
21 red, conical root, medium foliage
6 red, long root, medium foliage
8 red, round root, short foliage
31 red, conical root, short foliage
11 red, long root, short foliage
24 violet, round root, long foliage
52 violet, conical root, long foliage
17 violet, long root, long foliage
15 violet, round root, medium foliage
43 violet, conical root, medium foliage
21 violet, long root, medium foliage
20 violet, round root, short foliage
57 violet, conical root, short foliage
18 violet, long root, short foliage
11 white, round root, long foliage
23 white, conical root, long foliage
9 white, long root, long foliage
8 white, round root, medium foliage
19 white, conical root, medium foliage
12 white, long root, medium foliage
5 white, round root, short foliage
25 white, conical root, short foliage
13 white, long root, short foliage

417

Icicle × French Breakfast

French Breakfast has short-cylindric red roots and Icicle long-cylindric white ones. The very uniform F_1 has a broad-conical shape and a violet to purple color. When individuals, however, are allowed to be self-pollinated they produce, not only, as was expected, offspring with red, violet or white, short-cylindric, broad-conical or long-cylindric roots, but they also produce individuals of a pink color, which are presented in all shape types. The combinations appeared in the numbers shown in table 3.

TABLE 3

COLOR	FRENCH BREAKFAST FORM	ICICLE FORM	HETEROZYGOUS BROAD CONICAL	TOTAL
Red with white points	4	2	6	12
Pink with white points	3	1	2	6
Heterozygous violet with white points	38	28	52	118
White	6	3	9	18
Totals	51	34	69	154

These data do not come as close to a trihybrid ratio as might have been expected, owing to the considerable excess of violet; but there can be no doubt that this is the theoretical ratio involved.

Crosses involving Long White

Very interesting were the observations on the commercial variety, Long White. I found in a seed-bed of very young plants two distinct types as to the development of the color pigment, which can be detected only when the plants are very young, before the secondary cortex has developed and before the primary cortex of the root has disappeared. About 90 percent of the seedlings had white primary-cortex cells, but the rest showed red primary-cortex cells. As the roots become fleshy the red color disappears and there is then no difference to be observed. Therefore, these factors can be studied only when the seedlings are small.

I was able to obtain pure strains possessing each of these two alternative characters. Reciprocal crosses were made and all the F_1 plants showed roots with a reddish primary cortex. From self-pollinated plants I expected in the F_2 a ratio of 1 white to 3 red, but this was not always the case; where the red strain was used as mother I found 21 white and 232 red, and when the white strain was used as the mother there were 3 white and 34 red, i.e., ratios which approach 1 : 15. It is here evident that the red color is not due to a single factor, but is the result of two factors which may be called red$_1$ (R_1) and red$_2$ (R_2). Many F_2 individuals showed a splitting in the F_3 in 59 white and 188 red, others in 19 white and 51 red, thus closely conforming to a ratio of 1 : 3, whereas others split in 7 white and 118 red and in another case 29 white to 487 red, in which a 1 : 15 ratio can be easily recognized. On the other hand, a certain number bred true as to the red color pigment, but no attempts were made to determine in the latter, whether the formula was $R_1\,R_1\,R_2\,R_2$, $R_1\,R_1\,r_2\,r_2$, $r_1\,r_1\,R_2\,R_2$, $R_1\,R_1\,R_2\,r_2$ or $R_1\,r_1\,R_2\,R_2$.

Evidently two individuals with the color-factor combination $R_1r_1R_2r_2$ have produced the ratios 7 : 118 and 28 : 487, whereas those with $R_1r_1r_2r_2$ or $r_1r_1R_2r_2$ must have split in the ratio 59 : 188 and 19 : 51. There is no doubt that this cross behaved as a dihybrid, due to occurrence of two duplicate red-color factors.

Crosses involving Round Yellow

The Round Yellow radish, which is but very little grown, having round yellow roots and foliage of medium size, was hybridized with certain white varieties, especially Round White, Early White with short foliage,

and Icicle. There were all indications that the yellow pigment was due to only one differential factor. Reciprocal crosses between Yellow Radish and Round White, having the same foliage, gave in the F_2 35 white and 101 yellow radishes, a ratio of 1 : 3.

A cross between Round Yellow and Early White acted as a dihybrid. The F_1 was composed of individuals with yellow roots and a foliage intermediate between the two parent plants.

The F_2 was composed of 172 individuals, of which 140 were yellow and 32 white. Among the yellows I counted 34 with long foliage, 41 with short and 65 with medium-sized foliage, the last being regarded as heterozygous with respect to the foliage. Five of these were self-pollinated, and produced either long-, medium-, or short-leaved plants. Of the 32 white F_2 radishes there were 9 with long foliage, 5 with short and 18 with medium foliage, the latter being considered heterozygous.

Crosses between Round Yellow and Icicle gave rise in the F_1 to long-oval yellow radishes. Not enough offspring were obtained in the F_2; consequently sufficient data are not present to show a clear ratio, they were probably of a dihybrid nature. The following plants were noticed: 5 round yellow, 6 round white, 8 long-oval yellow, 5 long-oval white, 1 long yellow and 3 long white.

Crosses involving Black Winter

Another situation was offered when the Black Winter radish was used. The main characteristic of this group is the presence of a cork tissue around the periphery of the roots. In crosses with ordinary radishes this character is always dominant. The main factors in which the Black Winter radish differs from ordinary radishes are: (1) cork tissue; (2) shape of roots in some instances; (3) size of roots in all cases; (4) heavy foliage; (5) late maturity.

There are two distinct varieties of Black Winter radish, a round and a conical one, which when crossed with each other produced intermediates in the F_1 and 38 round, 72 intermediates and 27 conical ones in the F_2; consequently, they are of monohybrid nature.

Results obtained between the Black Winter radish and ordinary radishes were never sufficiently extensive to permit the determination of clear ratios, but in some cases there were, no doubt, from 4 to 6 factors to be considered.

A cross between Long Red and Black Round Winter was in the F_1 more or less conical. Through the black or grayish cork layer the red pigment due to Long Red could be easily observed in all individuals, of which 11

were obtained. The foliage was very heavy. From one plant 180 F_2 offspring were secured, of which 120 had the appearance of those of the F_1, 8 were like Black Round Winter, 12 had the shape of Long Red, but had the color of the F_1 and 23 were large, round and red. These data are clearly inadequate to give a proper idea of the actual ratio.

A cross between Yellow Vienna and Black Round Winter proved to be in the F_1 more or less roundish and black, but the yellow color due to Yellow Vienna, was everywhere visible. But 31 F_2 plants were grown, of which 18 were like the F_1, 9 had the appearance of Black Round Winter, and 4 resembled the Yellow Vienna. Here also a sufficient number of individuals was not present.

SUMMARY

This paper deals with factors for form and color of the roots, and length of foliage, in crosses between varieties of summer radishes, and between these and the black winter radishes.

In all crosses the form of the roots was determined in each case by a single factor pair and the heterozygote was intermediate between the two homozygous parents. The same thing was true in regard to length of foliage.

When white and red varieties were crossed, the heterozygotes were in every case violet to purple, easily distinguishable from the homozygotes.

A factor, *R*, for red striping gave 1 : 1 ratios following self-fertilization, suggesting the possible presence of a gamete lethal.

In the cross between white-rooted Icicle and red-rooted French Breakfast, there appeared in addition to the expected parental types and purple heterozygotes, a small percentage of pink-rooted plants.

In the variety Long White some seedlings had red primary-cortex cells while others had white. This difference is observable only in the early seedling stage. Some of the red seedlings bred true, others gave ratios of 3 red : 1 white, and still others 15 red : 1 white, indicating the occurrence of duplicate factors.

The yellow color of Round Yellow is due to a single factor difference from white, the yellow being dominant.

Black Winter radishes crossed with the summer radishes showed the corky tissue around the periphery of the roots of the former dominant over its absence, and the black color dominant or nearly completely epistatic over the other colors.

While many of the crosses produced too few offspring for a decisive determination of ratios, the results indicate that the radish varieties are worthy of extensive and intensive genetical analysis.

LITERATURE CITED

BARKER, E. E., and COHEN, R. H., 1918 Variability in the radish. Jour. Heredity 9: 357–361.
FROST, H. B., 1923 Heterosis and dominance of size factors in Raphanus. Genetics 8: 116–153.
HEGI, GUSTAV, 1921 Illustrierte Flora von Mittel-Europa. 4: 274–286.
TROUARD-RIOLLE, Y., 1920 Les hybrides de Raphanus. Rev. Gén. Bot. 32: 438–447.